

10.4

Large molecules in the structure and functioning of cells

Contents

Biological molecules

- ▼ Monomers and polymers.
- ▼ Condensation and hydrolysis

Carbohydrates

- ▼ Structure and properties of carbohydrates.
- ▼ The structure of alpha and beta glucose, and the linking of these monomers by glycosidic bonds
- ▼ Biochemical tests for reducing sugars, non-reducing sugars, starch
- ▼ The basic structure and function of starch, glycogen and cellulose

Proteins

- ▼ Amino acid structure and the peptide bond
- ▼ Primary, secondary, tertiary and quaternary structure of proteins
- ▼ The relationship of tertiary structure to function in globular proteins
- ▼ The biuret test for proteins

Lipids

- ▼ The structure of saturated and unsaturated triglycerides and phospholipids
- ▼ The emulsion test for lipids

Chromatography

- ▼ The technique of chromatography for separating and identifying molecules
- ▼ The calculation and use of R_f value
- ▼ Two-way chromatography

BIOLOGICAL MOLECULES

The bodies of living organisms are made up of fewer than 20 different chemical elements and just six of these bioelements make up 99% of the total body mass (oxygen 65%, carbon 18%, hydrogen 10%, nitrogen 3%, calcium 2%, phosphorus 1%). Most of the important biological molecules are organic, which means that they are made up from carbon compounds (note that most but not all compounds containing carbon

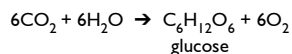
are organic; exceptions include carbon dioxide and carbonates). Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids. Individual molecules can act as sub-units (**monomers**) to combine by reactions called **condensation reactions**, in which a molecule of water is lost as each sub-unit joins the next. In this way monomers can form dimers (two joined monomers e.g. glucose and fructose forming sucrose), or chains of repeating sub-units (**polymers**). Bonds formed by condensation may be broken down by the reverse process, **hydrolysis**, which involves the addition of a molecule of water for each bond broken. The following account describes the structure of carbohydrates, proteins and lipids and the relationship of molecular structure to their function in living organisms.

CARBOHYDRATES

Structure and properties of carbohydrates

The structures of alpha and beta glucose

Carbohydrates are a family of molecules made up, as the name suggests, from carbon and the components of water (hydrogen and oxygen). Carbohydrates are manufactured by plants and are passed on to other organisms via food chains. You should already be familiar with the name and chemical formula of one of the simpler carbohydrate molecules, glucose, which is made from CO_2 and H_2O in the process of photosynthesis.

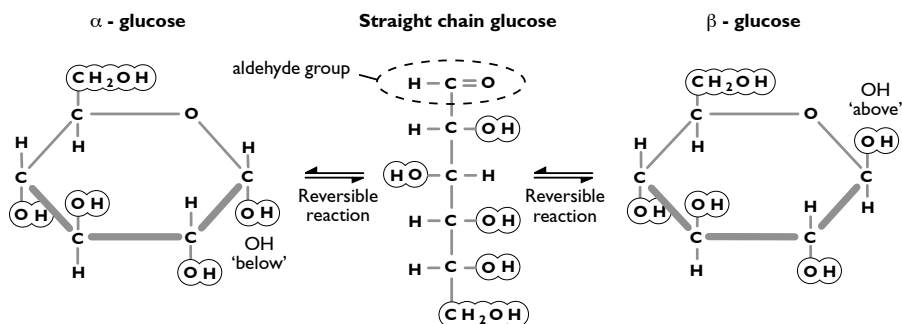


Glucose has six carbon atoms in the form of a framework to which the oxygen and hydrogen atoms are attached. In dry glucose powder the carbon atoms are arranged in a straight chain, but if it is dissolved in water, the chains form themselves into ring structures. Two different ring forms exist called **alpha glucose** and **beta glucose**. The carbon atoms are numbered 1 to 6 starting in a clockwise direction from the position of the oxygen atom. As discussed later, the different structures of alpha and beta glucose result in the formation of polysaccharides with different properties.

Alpha and beta glucose are **isomers**; they have the same chemical formula but a slightly different arrangement of atoms in the molecule (fructose and galactose are also isomers of glucose).

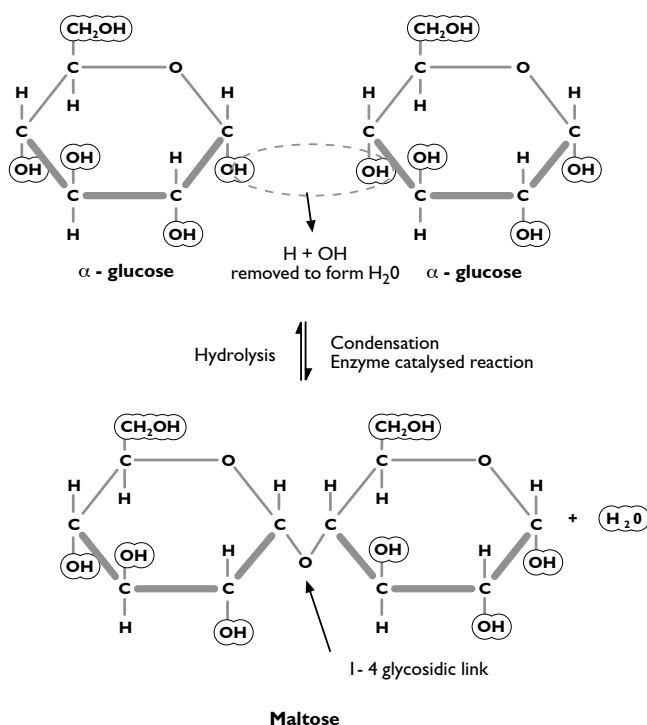
◆ CHECKPOINT SUMMARY

- ◆ Most of the important biological molecules are 'organic', which means that they are made up from carbon compounds
- ◆ Not all compounds containing carbon are organic. Exceptions include carbon dioxide and carbonates)
- ◆ Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids
- ◆ Individual molecules (monomers) can join to form dimers of just two monomers, or longer chains (polymers) of repeating monomers.
- ◆ Polymers are very large molecules, built up by reactions called condensation reactions in which a molecule of water is lost as each sub-unit joins the next
- ◆ Bonds formed by condensation reactions may be broken down by the reverse process, hydrolysis, which involves the addition of a molecule of water for each bond broken
- ◆ Condensation and hydrolysis reactions are catalysed by enzymes in the living cell
- ◆ Under laboratory conditions, in the absence of a catalyst, hydrolysis requires extreme conditions of temperature and pH.



The formation and breakage of glycosidic bonds

Ring structures like these are able to link together to form pairs or long chain molecules. The terms monosaccharide, disaccharide and polysaccharide are used to describe the number of glucose molecules making up a chain (mono = 1, di = 2, and poly = many). If the units of glucose link up to form larger molecules, the first carbon atom of one alpha glucose molecule links to the fourth carbon atom of another with the elimination of a molecule of water. This enzyme controlled reaction results in an oxygen bridge called a **glycosidic bond** (an alpha 1-4 glycosidic bond) and in the formation of the disaccharide **maltose**. The formation of more than one bond by condensation leads to longer and longer 'multi-molecules' or polymers.



Test for reducing sugars e.g. glucose

All the common simple carbohydrate sugars, with the notable exception of sucrose (table sugar), have the ability to **reduce** other chemicals. They are thus called reducing sugars and they can be identified by heating with **Benedict's reagent**. Benedict's reagent contains copper sulphate and has a clear blue colour. This changes to brick red as the copper is reduced to a solid precipitate of copper oxide in the presence of a reducing sugar. The quantity of reducing sugar in a positive test can be estimated from the amount of precipitate formed using a colorimeter or a standard colour comparison sequence.

Test for non-reducing sugars

Sucrose gives a negative result with the Benedict's test, but when heated to boiling with dilute hydrochloric acid it is hydrolysed into its constituent monosaccharides e.g. glucose and fructose, which when made alkaline and retested with Benedict's reagent give a positive result.

If a mixture of both reducing and non-reducing sugars are present, which would be highly likely with plant tissue extracts, the precipitate from the initial positive Benedict's test would need to be removed by filtration, and the resultant clear filtrate re-tested for sucrose as described above.

Structure and function of starch, glycogen and cellulose

Glucose is the universal fuel for respiration, but it cannot be stored in cells because a high glucose concentration would result in the entry of large amounts of water by osmosis which could cause cells unprotected by a cell wall or surrounding cells, to swell up and burst. For this reason, glucose in excess of energy requirements is converted for storage to the relatively insoluble polysaccharides **starch** in plant cells and **glycogen** in animal cells. These substances are similar in chemical structure, both being formed from chains of alpha glucose molecules.

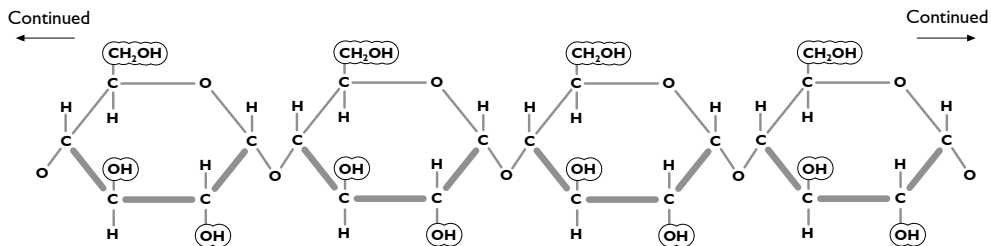
Starch is a mixture of **amylose** (unbranched chains of alpha glucose) and **amylopectin** (branched chains of alpha glucose). When combined together, the alpha glucose units have their oxygen bridges on the same side of the chain. Hydrogen bonds form between them and cause the chain to coil into a helix, so starch molecules have a dense, tightly packed structure and are relatively insoluble in water.

To dissolve starch, it is necessary to heat it. This breaks the hydrogen bonds and, as the coils begin to open, water molecules rush to occupy the exposed polar sites binding all over the surface. This is why starch suddenly swells in hot water and is the secret of starch based sauce thickeners used in cooking.

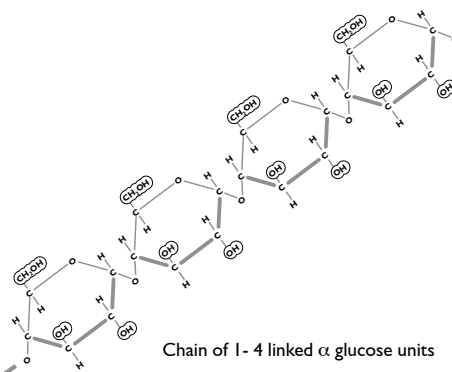
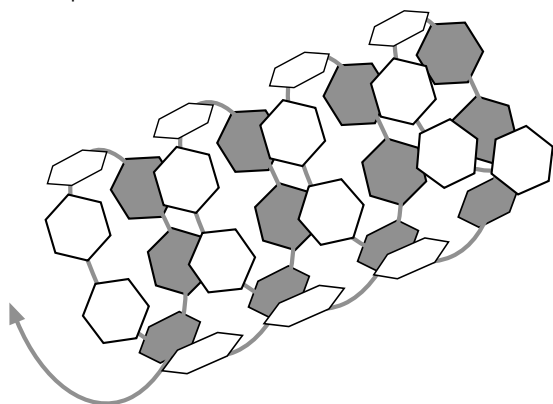
Test for Starch

A solution of iodine in potassium iodide is added to a solution of the crushed tissue at room temperature. The presence of starch is indicated by a colour change from light brown to blue-black.

Part of amylose molecule chain

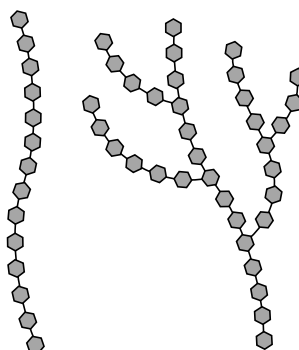


Hydrogen bonds between CH_2OH groups which are all on one side 'pull and twist' the chain to form a helix



Amylose - straight chains of glucose molecules

Amylopectin - branched chains of glucose molecules



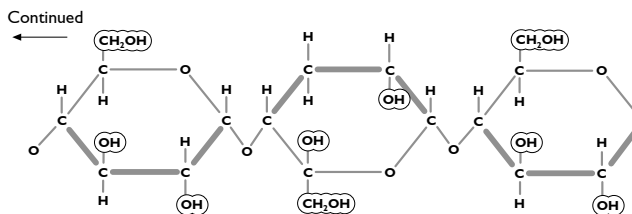
Glycogen has a similar structure to amylopectin but has a large number of shorter branches. It is more suited to animal metabolism as it is capable of being broken down more rapidly than starch.

Cellulose is the major component of plant cell walls. The characteristic fibrous structure of cellulose is created by chains of 1-4 linked beta glucose molecules. The beta molecules, when joined together, have their oxygen bridges on alternate sides of the chain, and thus it cannot form into a helix, but by forming hydrogen bonds with other chains lying side by side they make flat ribbons, which in turn form fibres.

The basic meshwork of cellulose fibres in plant cell walls is strengthened and cemented by other materials such as pectin and lignin. Cellulose is the main component of numerous textiles e.g. cotton, linen, hemp; timber products, paper and plastics e.g. celluloid.

Starch and cellulose have very different properties which arise as a result of one difference between alpha and beta glucose. Starch has no distinct structure (amorphous), no structural strength, is slightly soluble in water, and digestible by vertebrates. Cellulose has a distinct fibrous structure, great structural (tensile) strength, is insoluble in water and indigestible by vertebrates. (Herbivorous vertebrates harbour mutualistic microorganisms in specialised compartments of their gut which secrete cellulase enzyme which digests cellulose.)

Part of a cellulose molecule chain



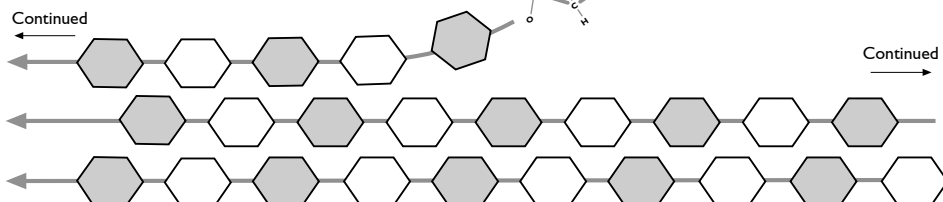
Notice how the CH_2OH groups alternate from side to side of the chain (the bonds indicated by a thick line are nearer)

◆ CHECKPOINT SUMMARY

- ◆ Simple formula for glucose - $\text{C}_6\text{H}_{12}\text{O}_6$
- ◆ Forms straight chain in dry powder form
- ◆ Forms ring structure in solution
- ◆ alpha and beta glucose are isomers differing in the 3D arrangement of their atoms
- ◆ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ◆ Glycosidic bonds are broken by the addition of the elements of water across the bond - a hydrolysis reaction
- ◆ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ◆ Both being long chains (polymers) of alpha glucose molecules
- ◆ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix
- ◆ Starch molecules are relatively insoluble in water
- ◆ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix
- ◆ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons which form cellulose fibres.

Arrangement of molecules in microfibril

Chains of 1-4 linked β glucose units lie parallel forming a microfibril



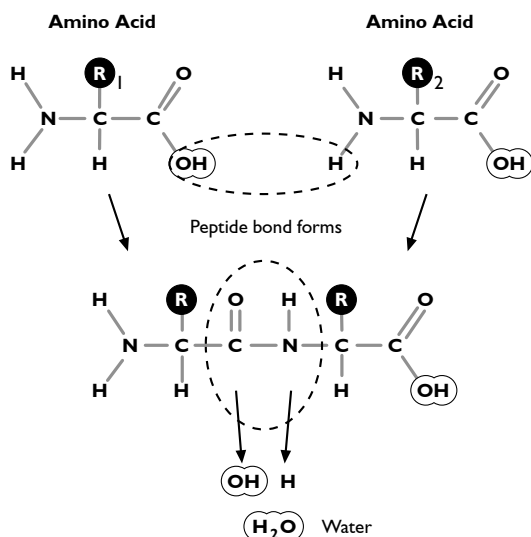
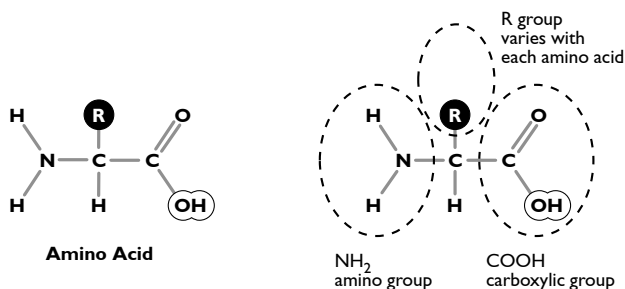
Hydrogen bonds form between alternating CH_2OH units bind molecules together side by side into microfibrils.

PROTEINS

Proteins are polymers made up of **amino acids**, joined together in long chains, which may be folded and twisted in various ways. There are as many as 100 000 different kinds of protein molecule in human cells. This variety of structure is made possible by the fact that the twenty different types of amino acid commonly found in proteins can be arranged in any order in chains up to 2000 units long.

Amino acids and peptide bonds

The chemical structure of an amino acid consists of a central carbon atom bonded to a hydrogen atom, a carboxyl group ($-\text{COOH}$), an amino group ($-\text{NH}_2$), and a side chain which varies with each amino acid, referred to as the 'R' group. **Peptide bonds** form between the amino ($-\text{NH}_2$) and carboxyl ($-\text{COOH}$) groups of adjacent amino acids to make chains called peptides (dipeptide = 2 amino acids linked together, polypeptide = many amino acids). As in carbohydrates, these linkages are made by a condensation reaction involving the elimination of a molecule of water.



Primary structure

The amino acid sequence is the starting point or primary structure of proteins. The final shape of proteins depends upon additional bonds which form between the $-NH$, $-C=O$, and $-R$ groups which 'stick out' of the sides of the polypeptide chain.

Secondary structure

The secondary structure of proteins is determined by hydrogen bonds between adjacent $-NH$ and $-C=O$ groups. Two very different formations are possible, the **alpha helix** and **beta sheet**. Both of these secondary structures may occur in the same protein

Tertiary structure

The tertiary (third level) structure of proteins is determined by bonds which form between R groups. There are three possible types depending upon the chemical properties of the different $-R$ groups. $-R$ groups may be acid, basic, polar or non polar.

Quaternary structure

The quaternary (fourth level) structure of proteins arises from the combination of separate polypeptide chains to form a larger complex, e.g. haemoglobin, which is composed of four polypeptide chains with iron containing haem units.

The relationship of tertiary structure to function

The types of bonding which hold protein molecules in shape determine the shape and properties of the protein.

Hydrogen bonds are formed between polar $-R$ groups (in addition to the H-bonds already described)

Ionic bonds are formed between the negatively charged acidic $-R$ groups and positively charged basic $-R$ groups. The forces are like those which exist between the Cl^- and Na^+ ions in salt.

Hydrophobic bonds occur between the $-R$ groups of non polar amino acids e.g. valine (Val) and leucine (Leu).

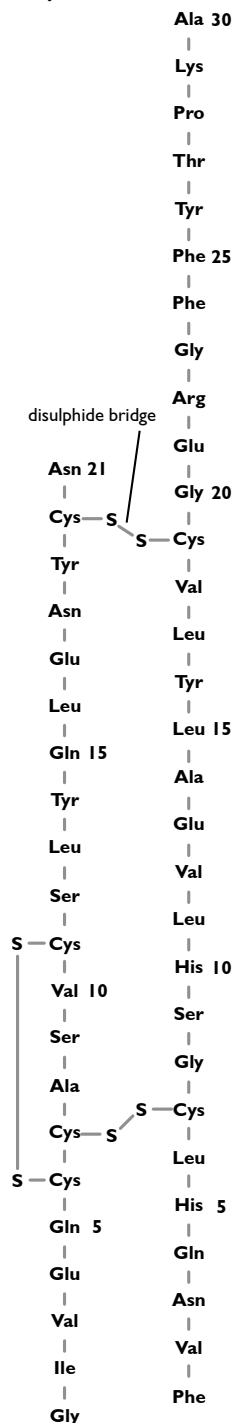
Disulphide bridges are much stronger covalent bonds formed between the sulphur atoms of molecules of the amino acid cysteine (Cys).

Proteins may be modified further by being linked to mineral ions, iron in the case of haemoglobin, or joined to carbohydrates and lipids to form **glycoproteins** and **lipoproteins**. This 'finishing off' process happens within the endoplasmic reticulum of cells.

Globular proteins

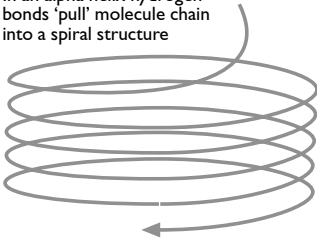
Globular proteins have a pronounced tertiary structure with many folds and twists, making them very susceptible to temperature and pH changes. Non polar groups tend to be repelled by water (hydrophobic) and tuck inside the molecule whilst the polar groups are attracted to water (hydrophilic) and they face outwards forming hydrogen bonds with water molecules. Globular proteins therefore tend to be soluble. Globular proteins are involved in metabolic activities e.g. enzymes, hormones, antibodies, and haemoglobin. **Haemoglobin** has a complex structure made up from four separate polypeptide chains (globins) joined to four iron (haem) ions. The iron (haem) groups in haemoglobin give the molecule its red colour and it is to these four sites that oxygen binds.

Primary structure

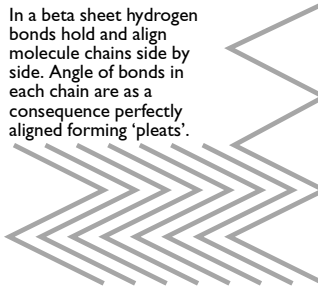


Secondary structure of proteins

In an alpha helix hydrogen bonds 'pull' molecule chain into a spiral structure

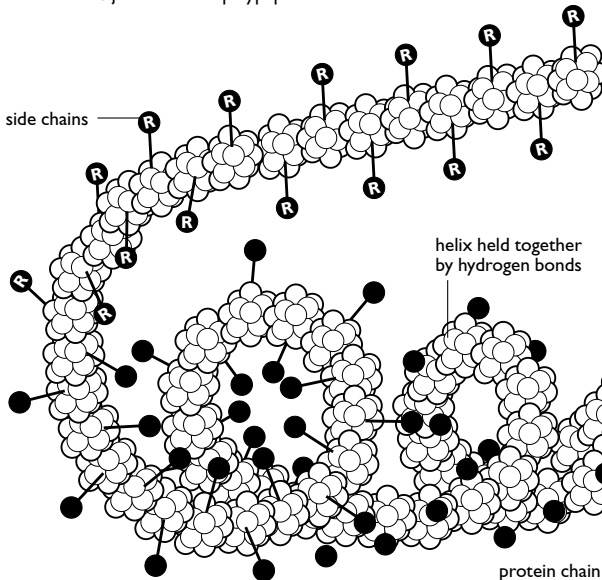


In a beta sheet hydrogen bonds hold and align molecule chains side by side. Angle of bonds in each chain are as a consequence perfectly aligned forming 'pleats'.



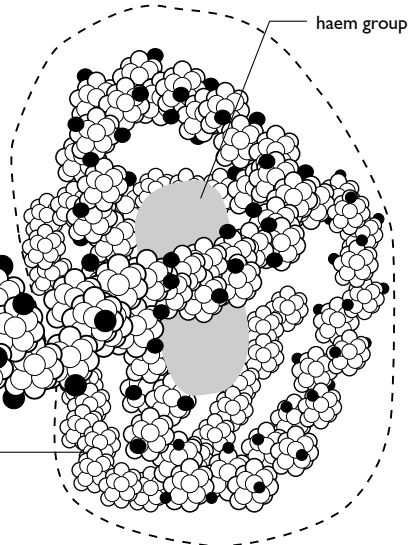
Primary structure

Amino acids join to form a polypeptide chain



Tertiary structure

Helix folds to form compact globular unit around Haem group



Secondary structure

Polypeptide chain folds in upon itself to form a helix

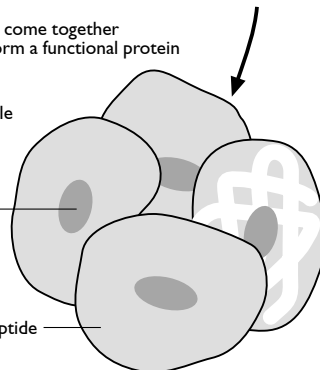
Quaternary structure

Several polypeptide chain units come together held by disulphide bridges to form a functional protein

Haemoglobin molecule has four subunits

haem group

globular polypeptide subunit



Fibrous proteins

In fibrous proteins the peptide chains are extended and inter-twined to form long thread like molecules. They do not dissolve in water and are relatively unaffected by changes in pH or temperature (they are not easily denatured). Fibrous proteins are mainly structural in function, giving support to tissues e.g. collagen in skin and bone, and keratin in skin and hair.

Test for Protein - the biuret test

2cm³ of dilute potassium hydroxide is added to 2 cm³ of the tissue extract in a test tube followed by a few drops of dilute copper sulphate solution. The presence of protein is indicated by a change in colour from light blue to purple (these colours may be interfered with by the colour of a food extract).

◆ CHECKPOINT SUMMARY

- ◆ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH₂), a carboxyl acid group (COOH), and a 'R' group, all attached to a carbon atom.
- ◆ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of CH₂NH₂COOH.
- ◆ Amino acids combine in a condensation reactions which form a peptide bond
- ◆ There are about 20 different types of amino acids found in proteins of living organisms
- ◆ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ◆ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.
- ◆ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins
- ◆ Hydrogen bonds between adjacent NH₂ and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein
- ◆ Bonds that form between the 'R' groups determine the tertiary structure
- ◆ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein
- ◆ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

LIPIDS

Lipids are a family of molecules which include fats, oils, waxes, phospholipids and steroids. Although they vary in chemical structure they share two important characteristics: they are energy rich substances, and they are insoluble in water.

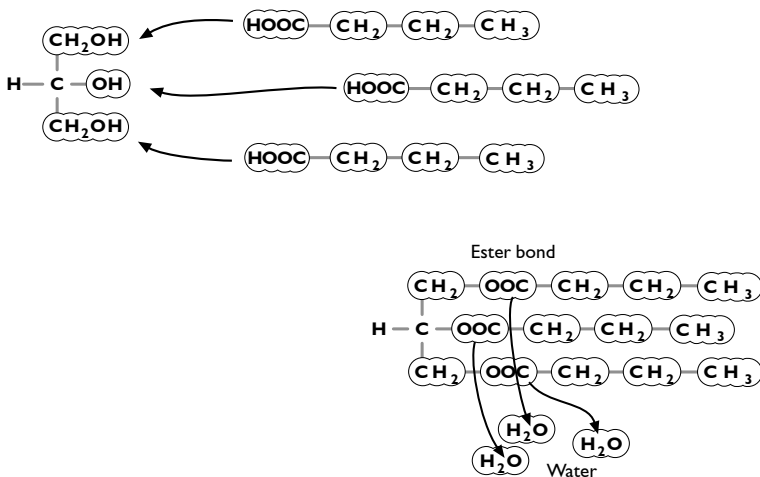
Structure and function of a triglyceride

Fats and oils are built on the same basic chemical plan which consists of a molecule of **glycerol** joined to three **fatty acid** chains. They are **triglycerides** ('mono' and 'diglycerides' are structures with one and two fatty acid chains respectively). The fatty acids are linked by **ester bonds** to glycerol in a condensation reaction similar to that seen in the formation of polysaccharides and polypeptides.

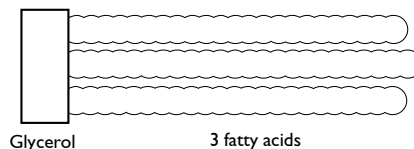
Fatty acids are hydrocarbon chains with a carboxylic ($-\text{COOH}$) group at one end. It is the $-\text{COOH}$ group which forms the ester bond with glycerol. Hydrocarbons are non polar and, like fuel oils which have a similar structure, they contain a lot of energy locked up in their C-C and C-H bonds. Fats are mostly of animal origin and are solid at room temperature (RT); oils occur mostly in plants and are liquid at RT. The difference in melting point is due to the different nature of the hydrocarbon chains. In fats, each of the possible bonding sites between carbon and hydrogen is fully occupied (they are **saturated**), but in oils, one or more double bonds occur between the carbon atoms in the chain (they are **unsaturated**). Wherever a double bond occurs, the fatty acid chain acquires a kink, so the fatty acid chains of oils occupy more space than those of fats, giving them greater mobility and a lower melting point so that they are liquid at RT. The terms 'mono', 'di', and 'polyunsaturated' refer to the number of double bonds in the fatty acids.

When more food energy is taken in by the body than can be used, it may be converted to fat which is stored in large **adipose** cells under the skin and around internal organs. Adipose tissue not only acts as a long term food store; it also insulates the body against rapid changes in temperature, gives mechanical protection to internal organs and, acts as a buoyancy aid in aquatic animals.

Molecular model of development of a triglyceride



Model of a triglyceride

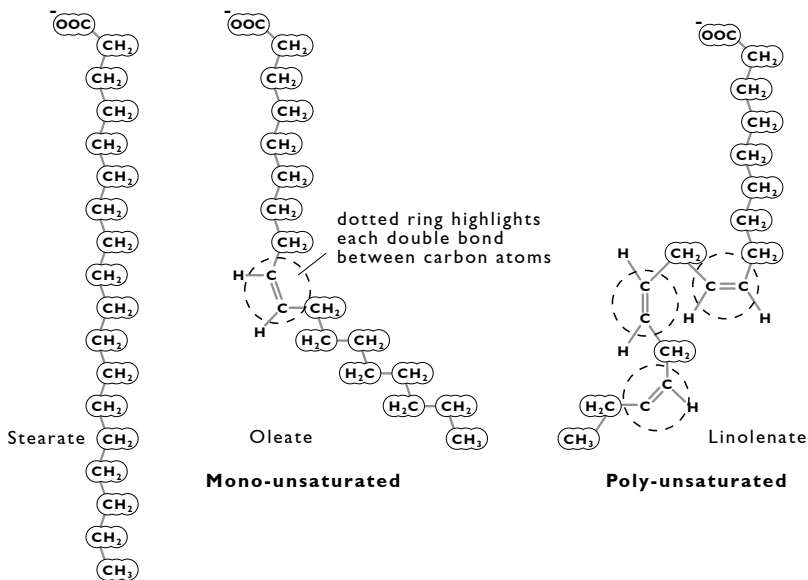


Waxes differ from fats and oils in that they have only one fatty acid chain and it is not joined to glycerol, but to a longer chain alcohol. They are produced in many animals and plants as a waterproof layer on external surfaces acting to reduce water loss (e.g cuticle of plant leaves, insect exoskeleton).

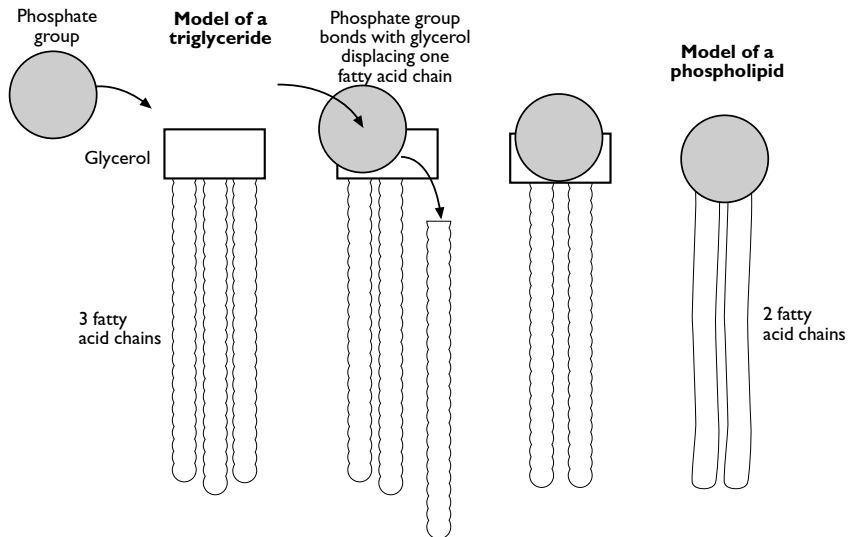
Structure and function of phospholipids

Phospholipids are essential to cells because they form the structural basis of all membrane systems. They have two fatty acid chains (which may be saturated or unsaturated) joined to a molecule of glycerol, the third position being occupied by a phosphate group (phosphate plus choline or ethanolamine).

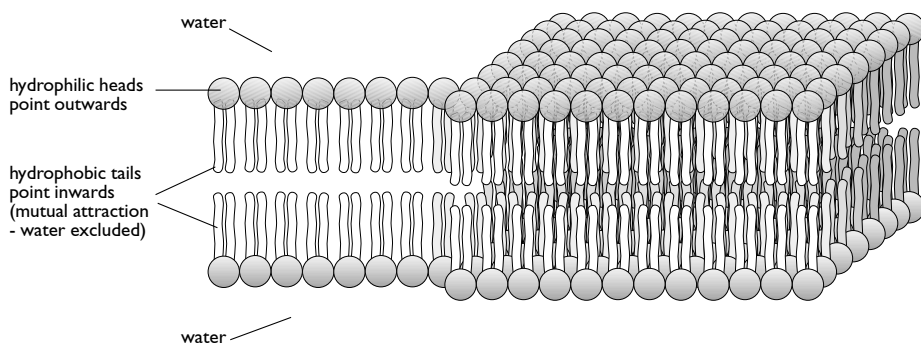
The phosphate group is strongly polar and readily dissolves in water, this is the hydrophilic (water loving) 'head' region of phospholipids. The non polar fatty acids are repelled by water and project in the opposite direction, and these are the hydrophobic (water hating) 'tails'. As a result, in the presence of water, phospholipid molecules form spontaneously into a characteristic **bilayer**. It is this bilayer which forms the molecular framework of all cell membranes.



Phospholipids



Phospholipid bilayer



Cholesterol is another important lipid component of cell membranes although it is very different in chemical structure to the lipids so far described. It has a ring structure and belongs to a group called **steroids** which include the sex hormones testosterone and oestrogen. Cholesterol molecules are strongly hydrophobic and they take refuge between the tails of phospholipids in membranes. Their presence in the bilayer has two effects. It slows down the lateral movement of individual phospholipid molecules, making the bilayer more stable, and it prevents the movement of certain substances through the bilayer, ensuring that membrane transport occurs through the correct (protein) channels.

Test for Lipids- the Ethanol Emulsion test

The tissue sample is ground in ethanol to dissolve any fats and oils. 2 cm³ of the filtered extract is then added to 2 cm³ of water in a test tube at room temperature. The presence of lipids is indicated by the formation of a milky white emulsion.

CHROMATOGRAPHY

The technique of chromatography to separate and identify molecules

The separation of different substances in a mixed solution can be achieved by chromatography or electrophoresis. These techniques exploit the fact that different molecules and ions tend to move at different rates through a paper or gel medium.

Paper Chromatography and R_f values

In this method, a concentrated sample of the mixture under study is formed by repeatedly placing small drops of it onto the same spot near one end of a piece of filter paper, letting the drop dry each time. The end of the paper is then dipped in a solvent solution such as ethanol and water. As the solvent front moves up the paper, so substances dissolved in it are carried along. The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper. The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a **chromatogram**, is allowed to dry.

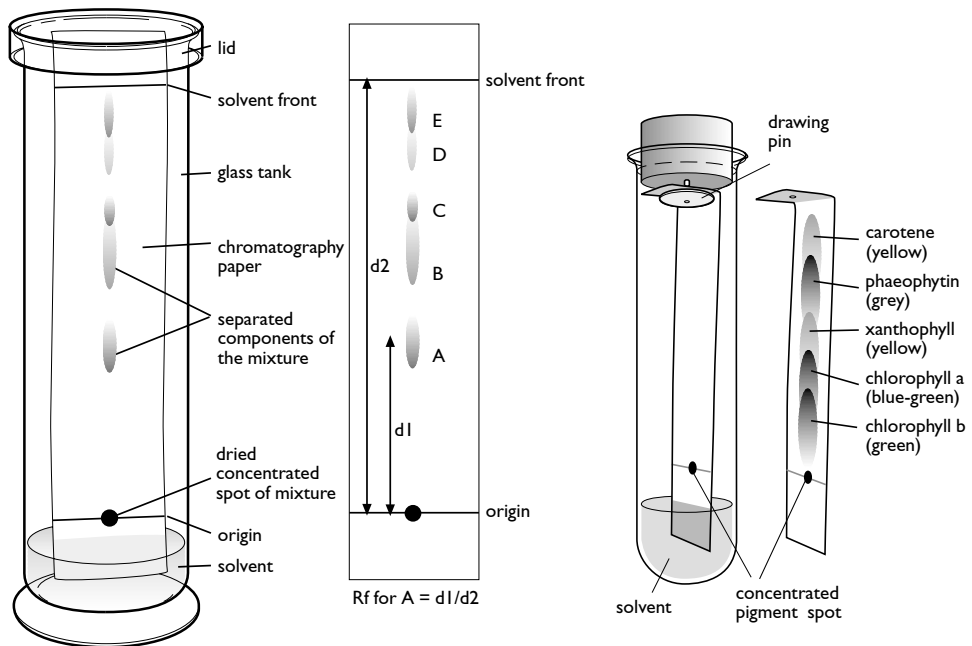
By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained.

$$R_f = \frac{\text{distance moved by the a substance from its origin (d1)}}{\text{distance moved by the solvent front (d2)}}$$

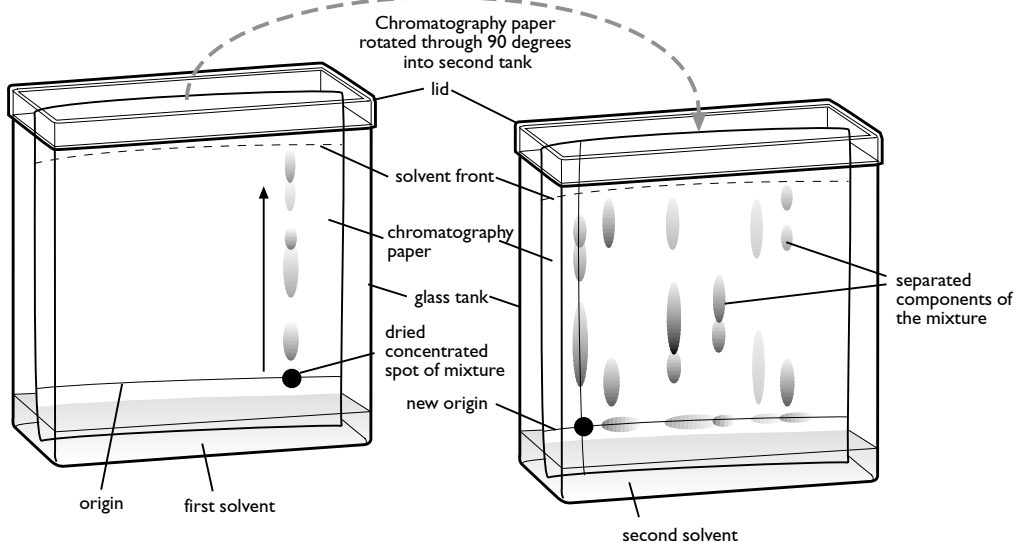
Two way chromatography

The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a **two way chromatogram**.

Separation of photosynthetic pigments



Two way chromatography



Thin Layer (gel) Chromatography

A slightly more sophisticated and faster version of paper chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above.

Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts, and the separation of amino acids from a protein sample. A concentrated sample of photosynthetic pigments can be obtained by grinding up leaves in a solvent such as acetone or ethanol. The different component molecules separate out in the chromatogram as clearly visible spots of different colours.

Proteins selected for this treatment must first be hydrolysed to break up the polypeptide chain into its constituent amino acids. This is done by soaking in hydrochloric acid for 24 hours. As amino acids have no colour of their own, they must be stained on the final chromatogram with a dye called **ninhydrin**. The colour intensity of each stained blob on the chromatogram can be measured using a colorimeter to reveal the proportion of each amino acid in the protein.

◆ CHECKPOINT SUMMARY

- ◆ Paper Chromatography is used to separate small traces of materials from a mixture
- ◆ A concentrated spot of the mixture is placed near one end of a piece of chromatography (or filter) paper. The end of the paper is then dipped in a solvent solution such as ethanol and water
- ◆ As the solvent front moves up the paper, so substances dissolved in it are carried along
- ◆ The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper
- ◆ The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a chromatogram, is allowed to dry
- ◆ By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained
- ◆ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram
- ◆ A slightly more sophisticated and faster version of chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above
- ◆ Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts.

Enzymes as proteins which control biochemical reactions in cells

Contents

Enzyme action

- ▼ The protein nature of enzymes
- ▼ Enzymes as catalysts lowering activation energy through the formation of enzyme-substrate complexes.
- ▼ The lock and key and induced fit models of enzyme action.

Factors which affect enzyme activity

- ▼ The effects of temperature, pH, substrate and enzyme concentration, competitive and non-competitive inhibitors.

ENZYME ACTION

The protein nature of enzymes

Most students of science can name an enzyme. Usually it is a digestive enzyme like amylase or pepsin, but enzymes are not just concerned with breakdown reactions like digestion, they can also help build up molecules. In fact, every chemical reaction in every living cell is made possible by an enzyme. Several thousand enzymes have been isolated and studied.

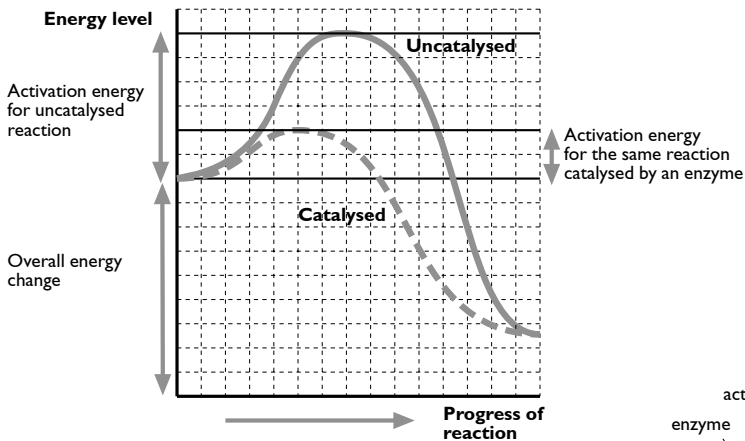
Enzymes are protein molecules. Compared to many other molecules they are relatively large (macromolecules) of the globular type described in 10.4 with a complex tertiary structure. What gives each enzyme its particular qualities and mode of action is the way in which its polypeptide chains are folded and twisted into specific shapes. As you will see in the following account, enzyme action depends upon shape recognition between enzymes and reacting molecules called **substrates**.

Enzymes as catalysts lowering activation energy through the formation of enzyme-substrate complexes

Enzymes are **biological catalysts**. A catalyst is a substance which speeds up a reaction without being changed itself in the process. As they are not used up or changed, enzymes can be used repeatedly, therefore they are effective in relatively small amounts.

Usually enzyme molecules are much larger than the reacting molecules (substrates). In an enzyme-catalysed reaction, the substrate(s) become attached to a particular region on the enzyme called the **active site** which has a specific shape and structure matching that of the substrate(s), forming the **enzyme - substrate complex**. This association is temporary and lasts only long enough to allow the substrate molecules to interact to form the products. Once the products are formed, they are released from the active site and the enzyme is free to repeat the process.

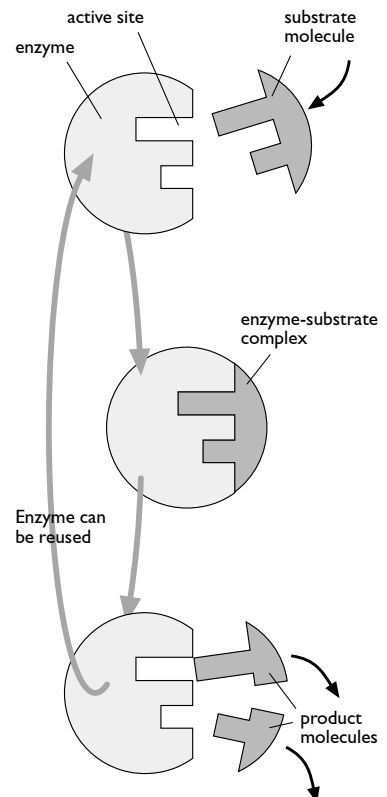
The formation of the enzyme-substrate complex lowers the **activation energy**, which is the energy needed to start a reaction. In the laboratory, it is common for reacting substances (substrates) to be heated in order to make the reaction occur more quickly. The heat energy causes the molecules to move about more rapidly (i.e. it gives them more kinetic energy) so that they collide more frequently, forming the product. In other words heat is used to reduce the activation energy. Living cells operate at a constant relatively low temperature so therefore they rely on enzyme catalysts to overcome the activation energy.



The lock and key and induced fit models of enzyme action

The active site matches the substrate, so different shaped substrate molecules require different shaped active sites. Most biological reactions therefore involve different, **specific** enzymes. This is possible because proteins which make up enzymes can be made in an infinite variety of shapes as a result of the nature and sequence of the amino acids and their R group (primary structure) which in turn leads to the development of a complex 3D structure (secondary, tertiary and quaternary structures).

This model of enzyme action is referred to as the **lock and key model**, but it is known that the shape of the active site may change as the substrate makes contact with it, adjusting to make a close fit, rather like a soft glove around a hand, a mode of action called **induced fit**.



FACTORS which affect ENZYME ACTIVITY

Temperature

Temperature affects the rate of all chemical reactions. As the temperature increases, so does the kinetic energy of the reacting molecules. More enzyme-substrate complexes are formed as the reactants collide more frequently, and the reaction rate rises exponentially, doubling for every 10°C rise in temperature. However, enzymes, like all proteins, suffer irreversible alterations in molecular shape above certain temperatures as a result of the breaking of chemical bonds within the molecule, so that in enzyme-catalysed reactions, there is an **optimum** temperature above which the reaction rate drops sharply. For an enzyme, any change in the shape of the active site means a loss of function, the enzyme is said to be **denatured**. For most enzymes, the optimum temperature is around 40°C, but there are extreme exceptions. For example, some bacteria living in hot springs have enzymes which function at temperatures above 85°C, whilst the ice fish of the Antarctic possess enzymes which operate at -2°C.

pH

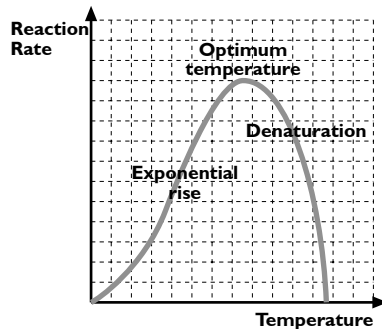
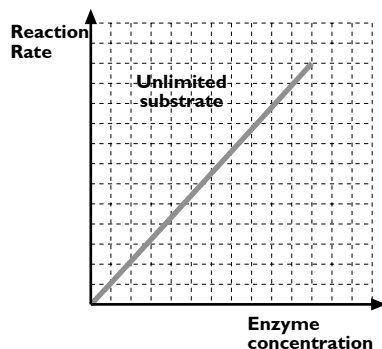
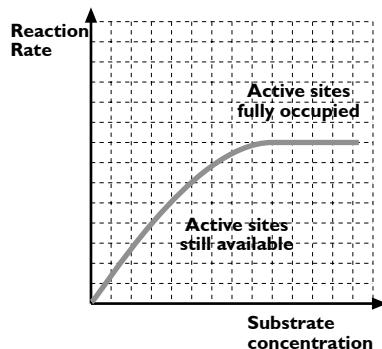
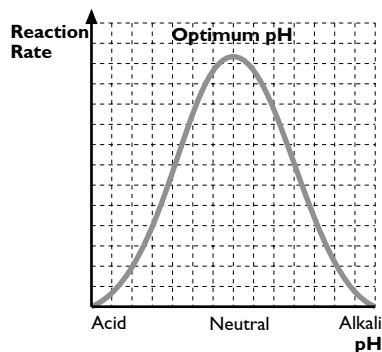
This is a measure of hydrogen ion (H^+) concentration, which is a measure of acidity and alkalinity. A pH of 7 is neutral, less than 7 is acid, and more than 7 is alkaline. Small changes in pH can affect the association process between enzyme and substrate which happens at the active site. The attraction between substrate and enzyme is often the result of small electric charges at the active site, and these are disrupted by changes in H^+ ion concentration. Extremes of pH, i.e. very acid or alkaline conditions, cause permanent denaturation. Enzymes have an optimum pH for efficient functioning. Usually this is around pH7, the normal pH of cells. Notable exceptions are the enzyme pepsin, which is found in the stomach and works best in highly acidic conditions in the range pH 1-2, and the enzyme trypsin which is found in the duodenum and has an optimum pH of 9.

Substrate concentration

Substrate concentration exerts a direct effect on the rate of the reaction. At low substrate concentrations, not all the active sites will be occupied, so the rate of the reaction depends upon the concentration of substrate alone. As the concentration of substrate increases, so all of the available active sites will become filled. The upper limit is determined by the amount of enzyme molecules available.

Enzyme Concentration

Enzyme concentration has a similar effect. As long as there is a plentiful supply of substrate, any increase in the number of enzyme molecules will result in a proportional increase in the number of enzyme-substrate complexes and therefore an increase in the rate of reaction.



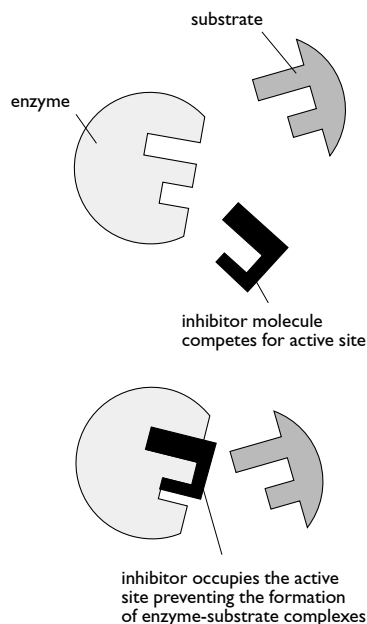
Enzyme Inhibitors

Enzyme inhibitors are substances which interfere with the active site of the enzyme. They do this either directly by blocking it, or indirectly by attaching to another part of the enzyme and altering its molecular shape so that the enzyme-substrate complex cannot be formed.

Competitive inhibitors

These are substances which have a molecular structure resembling that of the substrate. They attach themselves to the active sites forming inhibitor-enzyme complexes and cause the reaction to slow down or stop altogether. The effect of competitive inhibitors depends upon the relative concentrations of the substrate and inhibitor, and also the relative stability of the enzyme-substrate and inhibitor-enzyme complexes. This type of inhibition is seen in the action of a group of antibiotics called sulphonamides, which compete for the active site of a key bacterial enzyme involved in synthesising an essential growth factor, not found in the cells of the host.

Competitive inhibitor

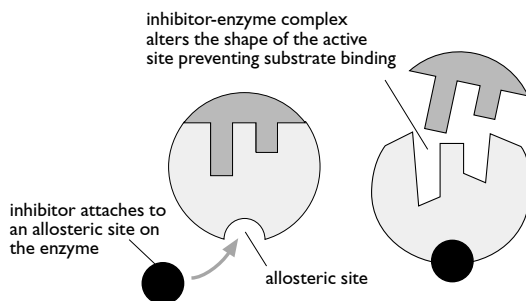


Non-competitive inhibitors

These are substances which bind onto the enzyme at a place other than the active site called an **allosteric site**, causing the enzyme to change shape sufficiently to stop enzyme-substrate complexes being formed. Non-competitive inhibitors are not similar to the substrate molecules and are not affected by the substrate concentration. The ions of some heavy metals such as arsenic and cyanide may bind onto allosteric sites and act as poisons, stopping important enzyme controlled reactions. Some enzyme molecules possess an allosteric site which must be occupied by an **activator** substance to give the enzyme its correct working shape. Without the activator, the enzyme will not work.

Inhibitors and activators are important in regulating the activity of enzymes.

Non-competitive inhibitor



◆ CHECKPOINT SUMMARY

- ◆ All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure
- ◆ Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process
- ◆ Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.
- ◆ Their activity depends upon their complex 3D shape which creates an active site which combines with the substrate to form an enzyme/substrate complex which lowers the activation energy of reactants
- ◆ Their complex 3D shape also accounts for enzyme specificity as the active site has a shape complementary to the shape of the substrate
- ◆ Disruption of the 3D shape accounts for denaturation.

Genetic code

Contents

DNA as genetic material

- ▼ Evidence that DNA is the genetic material
- ▼ The structure of DNA, mRNA and tRNA in terms of nucleotides, base pairing and hydrogen bonding.

Nucleic acids and DNA replication

- ▼ The roles of nucleic acids in coding information, protein synthesis and the replication of DNA
- ▼ The semi-conservative replication of DNA

Protein synthesis

- ▼ The genetic code as a non-overlapping, degenerate code. Introns as non-coding DNA.
- ▼ The mechanism of protein synthesis involving the roles of mRNA, tRNA and the ribosomes.
- ▼ DNA determines the structure of enzymes which determine the phenotype of an organism

DNA as GENETIC MATERIAL

Evidence that DNA is the genetic material

DNA (deoxyribonucleic acid), is so named because it is an acid and it is found in the nucleus. It is a polymer made up of repeating sub-units, in this case, nucleotides linking up to form a polynucleotide. What makes DNA so important and so unique is that it contains within its structure the genetic code, and it can produce copies of itself (replicate).

The nucleus of cells contains mostly DNA and protein. For many years it was thought that protein was the hereditary material, but when it became possible to extract and measure the quantity of DNA, it was found that the DNA content doubled in cells just before dividing. It was also found that, in the formation of sex cells, the amount of DNA halved. Both pieces of evidence underline its central role in inheritance. Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.

The structure of DNA and RNA

DNA is a polynucleotide made up of repeating nucleotide units. A **nucleotide** consists of three main parts: a pentose (5 carbon) sugar, joined to a phosphate group, and a nitrogen-containing organic base.

There are four different types of nucleotide in DNA each with a different organic base. The bases are **adenine** (A) and **guanine** (G) which belong to a group of chemicals called **purines**, and **cytosine** (C) and **thymine** (T) which belong to group called **pyrimidines**. Chains of nucleotides link up by condensation reactions, with the sugar and phosphate molecules forming the 'spine' of each strand of the molecule, with the bases to one side.

DNA consists of two such chains or strands linked by **hydrogen bonds** which form between pairs of bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure known as the double helix. Hydrogen bonds form only between specific pairs, namely A with T and C with G. The strands are therefore **complementary** to each other. The sequence along one strand determines the sequence of the other (complementary) strand. The understanding of the structure of the DNA double helix was one of the most significant breakthroughs in Biology.

RNA (Ribo(se)nucleic acid), like DNA, is a polymer made up of repeating nucleotides. It differs from DNA in the following three ways.

RNA is single stranded, not double stranded.

The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom).

The pyrimidine base Uracil (U) is found instead of Thymine (T).

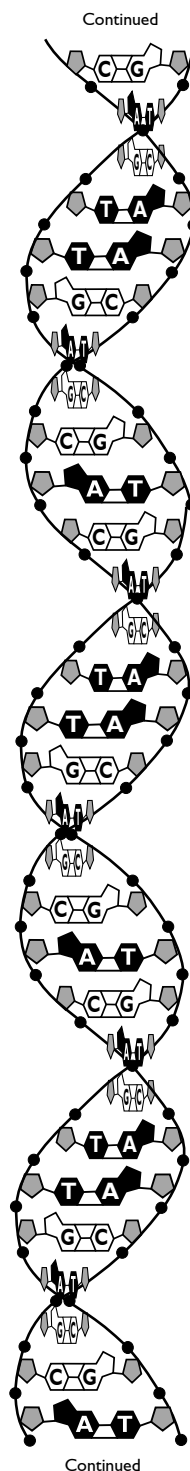
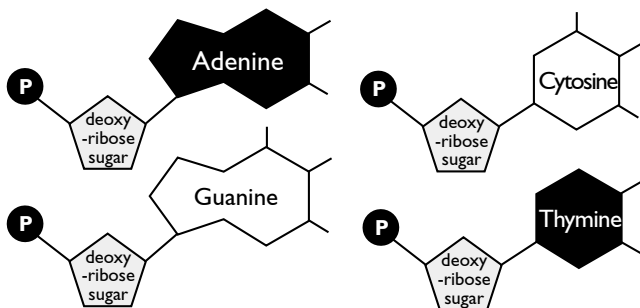
Living cells produce three different types of RNA in their nuclei, each designed to carry out a specific function in protein synthesis.

Messenger RNA (mRNA) consists of a single strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section of the DNA code. The mRNA molecules travel out of the nucleus to the ribosomes where proteins are synthesised.

Transfer RNA (tRNA) is a shorter length of RNA, between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the anti-codon which attaches to mRNA at the site of protein synthesis. tRNA molecules bring amino acids to their correct position as the proteins are being assembled. Each type of amino acid is carried by a different type of tRNA molecule with a specific anti-codon.

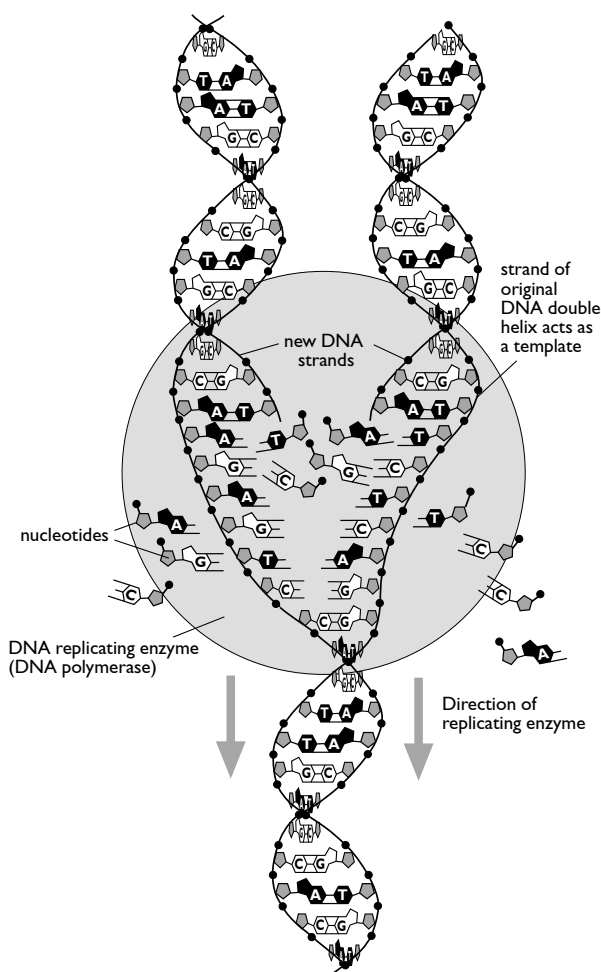
Ribosomal RNA (rRNA) consists of more than 1000 nucleotides and is found in ribosomes which are composed of an approximately equal mixture of proteins and rRNA. Ribosomal RNA is manufactured within the nucleolus.

The Nucleotides of DNA



Replication of DNA

In the process of DNA replication the original strands separate, and each acts as a pattern (template) for the formation of another new strand of DNA. Each of the daughter DNA molecules retains (conserves) one of the original strands and has one new one. For this reason, the process of replication is said to be **semi-conservative**. The process requires a supply of the four different nucleotides, energy in the form of ATP, and an enzyme called DNA polymerase, which is required to catalyse the condensation reactions by which free nucleotides form a copy of the template strand.



◆ CHECKPOINT SUMMARY

- ◆ The nucleus of cells contains mostly DNA and protein
- ◆ The DNA content doubles in cells just prior to dividing
- ◆ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underlines its central role in inheritance
- ◆ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering
- ◆ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides
- ◆ Nucleotides consist of a 5 carbon sugar; a phosphate, and an organic base
- ◆ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine
- ◆ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine
- ◆ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine
- ◆ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine
- ◆ There are three types of RNA - messenger; transfer and ribosomal
- ◆ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Experimental evidence for the semi-conservative mechanism of DNA replication includes experiments in which the mass of the DNA content of *E. coli*, grown on different nutrient media was determined and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and compared with that of organisms cultured on a medium containing normal 'light' nitrogen (^{14}N). The *E. coli* cells grown on 'heavy' nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was found to be halfway between the 'heavy' and 'light' values, indicating that half the DNA was old and half was new (semi-conservative replication).

The genetic code

The DNA base sequence forms a four letter language (A,G,C,T) which, as you have seen, can be copied every time a cell divides. This DNA base sequence determines the sequence of amino acids in a polypeptide chain (the primary structure of proteins). A sequence of bases which codes for one polypeptide is known as a gene. Each gene codes for a different polypeptide chain. The primary structure of a polypeptide is the basis of the particular structural shape and characteristic of the final protein, e.g. enzymes, structural materials and membrane proteins. The base code determines the structure, functions and individuality of every organism.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter base language (A,G,C,T). A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala) whilst 'GCT' codes for arginine (Arg), so the code 'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

polypeptide. Note that the code is **non-overlapping**, i.e. it must be read 'CGC-GCT-CGC', not 'CGC-GCG-CGC-GCT-CTC-' etc.

Sixty four different triplet codes (codons) are possible with this four letter base language, more than sufficient to code for the twenty different types of amino acids. The term **degenerate** is applied to the genetic code because there are more codons available than can be used to code for amino acids. In fact most amino acids are coded for by more than one codon each. The process by which this code is used in protein synthesis, is described below.

Protein Synthesis

Protein synthesis involves all three of the RNA types described above working in combination, and is achieved in two stages. As the DNA cannot leave the nucleus, first the code has to be read and copied (transcribed) into mRNA, in a process called **transcription**. The mRNA moves out of the nucleus and associates with the ribosomes, where the code (now embedded in the mRNA) is used in the synthesis of a polypeptide in the process of translation.

Transcription begins as an enzyme, **RNA polymerase**, attaches to the '**Start**' codon of a gene. The DNA base sequence of this 'Start' codon is always 'TAC'. As RNA polymerase attaches to the DNA, the hydrogen bonds binding the two strands of the DNA double helix break apart to expose the sequence of triplet base codons on both strands. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA. As it does so, a molecule of mRNA is formed copying one of the DNA strands. Only one of the two DNA strands (the template strand) is copied into mRNA. The mRNA is therefore an exact replica (save that 'U' replaces 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the 'Stop' codon of the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where the next stage occurs.

A complicating feature of the process of transcription in eukaryotic cells (cells with a nucleus) is that the length of DNA to be copied into mRNA (the gene) has sections of '**junk code**' which do not code for any of the required amino acids in a polypeptide chain. The 'junk' sequences are called **introns** and the parts to be transcribed and translated are called **exons**. The whole length is transcribed into mRNA, but before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, 'unpolluted', strand.

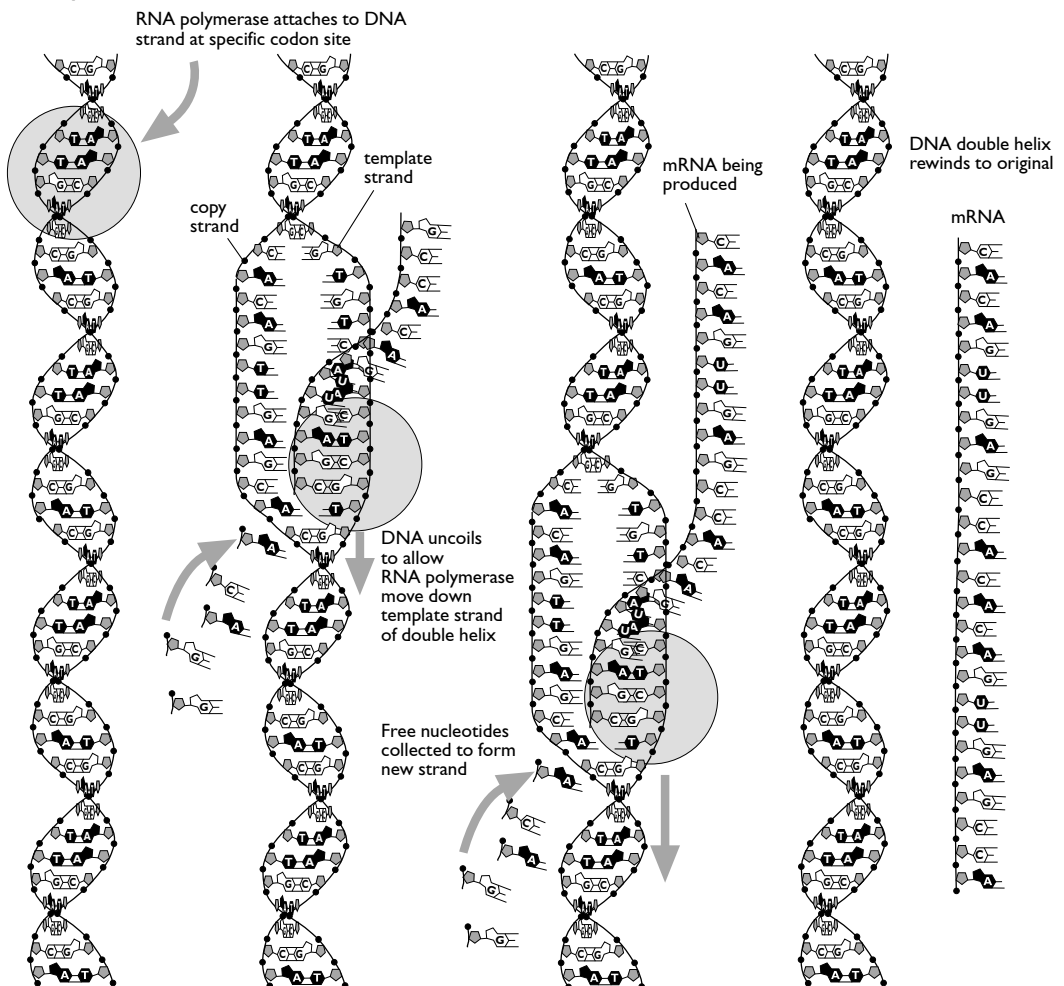
Translation occurs at ribosomes which are made up of two sub-units, one larger than the other, which come together only for the purpose of translating a length of mRNA. The mRNA binds to the small sub-unit at its 'Start' codon (AUG). It is then joined by the large sub-unit along with a molecule of tRNA which has the anti-codon 'UAC' and which carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can then proceed.

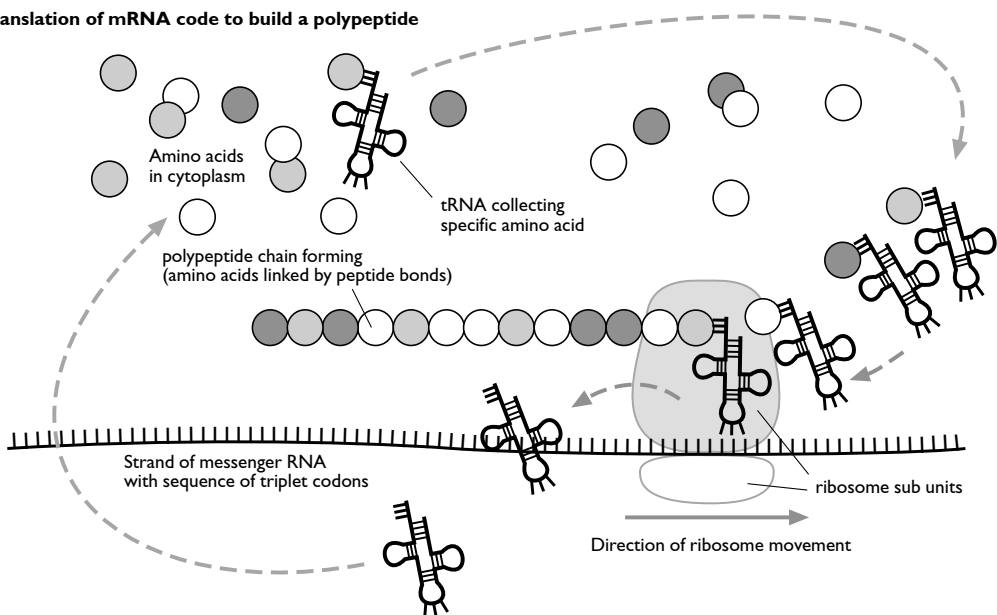
A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two amino acids. The ribosome moves along the length of the mRNA, and

once a tRNA molecule has delivered the appropriate amino acid it is released to repeat the process. New tRNA molecules with their amino acids come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the **'Stop' codon** (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

Generally mRNA molecules only have a short existence, sometimes just a few minutes, so that if continuous supplies of a particular protein is required the above processes must be repeated.

Transcription



Translation of mRNA code to build a polypeptide

◆ CHECKPOINT SUMMARY

- ◆ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells
- ◆ Three bases (a triplet codon) code for one amino acid
- ◆ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for
- ◆ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'
- ◆ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide
- ◆ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ◆ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon
- ◆ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood
- ◆ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells
- ◆ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes
- ◆ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ◆ There are 20 different types of tRNA, each specific to a particular amino acid
- ◆ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid
- ◆ The amino acids combine by means of peptide bonds to give a polypeptide chain
- ◆ The polypeptides subsequently form proteins
- ◆ As enzymes are proteins their synthesis is controlled by DNA.

DNA control of enzyme synthesis

Since the base sequence of DNA in a gene controls the production of one specific polypeptide or protein, it follows that any change in this base sequence (a gene mutation) may lead to the production of a faulty protein. Even a substitution or deletion of a single base can alter the translation of the DNA code into a sequence of amino acids in a polypeptide chain. If the primary structure of a polypeptide is altered this can affect its secondary, tertiary and quaternary structures, with corresponding effects on its function. The presence of such a faulty protein may in turn affect the appearance and functioning (phenotype) of the organism as a whole and cause a genetic disease or disorder. Gene mutations which occur during the production of gametes are thus the cause of genetic diseases in the offspring.

Some genetic diseases are referred to as 'inborn errors of metabolism' because they affect one or more of the metabolic pathways involved in the normal functioning of the organism. They may, for example, include diseases where particular enzymes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU). In this example the pathway from faulty gene to abnormal phenotype is clear. Identification of the gene and protein product responsible for the disease can allow medical intervention at any point in the pathway from gene to disease.

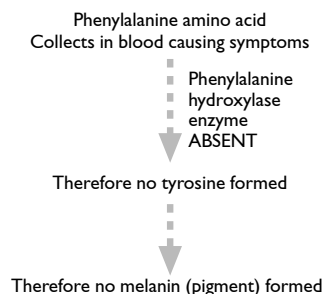
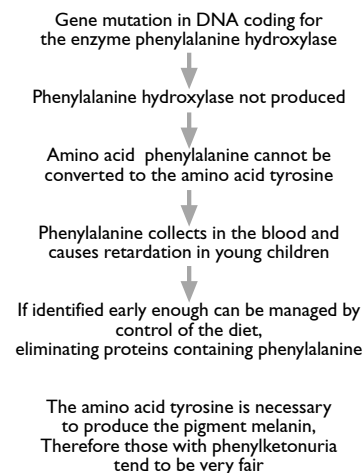
Phenylketonuria (PKU)

Most humans are able to convert some types of amino acids into other types of amino acid in the liver and thus produce all the proteins necessary for growth, repair and functioning of tissues. This explains why some amino acids are said to be essential (must be taken in the diet) whilst others are non-essential (do not need to be taken in the diet as they can be made from the essential amino acids in the body). Conversion of one amino acid to another (transamination) allows the body to make full use of ingested proteins and also prevents the build up of particular unused amino acids which could be dangerous.

In the disease phenylketonuria (PKU), however, a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver. Without phenylalanine hydroxylase the body cannot convert the amino acid **phenylalanine** to the amino acid **tyrosine**. This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.

PKU can be treated by modifying the diet to ensure that it lacks, or is very low in, the amino acid phenylalanine. The amino acid tyrosine may also be given. Under these circumstances individuals will not develop any signs of retardation. In the UK, all new-born babies are screened for PKU using a simple blood test which allows individuals with this genetic condition to be detected and treated before damage occurs. Treatment is only necessary in children, as adults seem to be unaffected by the high phenylalanine levels.

PKU sequence and effects



◆ SUMMARY OF PKU

- ◆ Gene mutation occurs in DNA coding for the enzyme phenylalanine hydroxylase
- ◆ Enzyme phenylalanine hydroxylase not produced
- ◆ Faulty metabolic pathway: amino acid phenylalanine not converted to tyrosine
- ◆ Phenylalanine accumulates with toxic effects
- ◆ Abnormal phenotype: Mental and physical retardation occurs.

Gene technology

Content:

Recombinant DNA

- ▼ The production of recombinant DNA and its use in the production of human insulin and other proteins
 - isolation of the required gene
 - use of the enzymes: reverse transcriptase, restriction endonuclease and ligase
 - sticky ends: insertion of the gene into a vector and its subsequent introduction into host cells; plasmids and viruses as examples of vectors
 - use of genetic markers such as genes conferring antibiotic resistance to detect genetically modified organisms
 - multiplication of host cells.
- ▼ The moral and ethical issues associated with recombinant DNA technology.

RECOMBINANT DNA

Imagine how simple life would be if there were only one type of computer and only one type of software. All the machines could then use the same language and all the information would be interchangeable. This is exactly how it is with the language of the genes. The synthesis of polypeptides from amino acids relies on exactly the same mRNA codons in every living organism so, for example, 'GCG' codes for the amino acid alanine and 'CGA' codes for arginine whether it occurs in a human, a honey bee or a mushroom. Now that genes can be identified and isolated, gene technology is providing a means for transferring them from one organism to another.

The terms 'genetic modification', 'genetic engineering' and 'gene technology' refer to the processes and techniques by which genes may be extracted from the DNA of one organism and inserted into the DNA of another organism (the **host** organism). In this way, the gene set (**genome**) of the host can be **transformed** (genetically modified). The modified DNA is called **recombinant DNA** because it has been re-combined to make a different set of codes.

Examples of genetically engineered products

Insulin is needed by people who are unable to produce sufficient quantities of the hormone and who would otherwise develop the disease **diabetes mellitus**. This is a condition, affecting 2% of the population, in which blood sugar rises to dangerous levels. Before gene technology, the only source of insulin was natural. It was extracted from the pancreases of pigs and cattle. This was not ideal because human insulin is slightly different in chemical structure, and although the pig and cattle version works on glucose in a similar way, some people are allergic to it and the process is both costly and open to contamination by impurities.

In the early 1980s the gene for human insulin was transferred from human pancreas cells to *E. coli* bacteria. Bacterial colonies modified in this way were cultured for mass production in industrial fermenters providing a constant supply of insulin. Now most insulin is produced by transformed bakers' yeast cultures. Yeast has various advantages over bacteria. It does not produce any toxic by-products, it can be grown on simple nutrient media, and it can secrete the finished product directly into the culture medium making the harvesting process easier.

Factor VIII is a vital blood clotting factor lacking in people suffering from **haemophilia**. Natural sources are both expensive and liable to contamination but now the gene for factor VIII has been engineered into yeast cells.

The food industry uses more and more genetically engineered products. One of the first to be developed on a commercial scale was the enzyme **chymosin** (rennilase) which is used to coagulate milk for the production of cheese. Chymosin occurs naturally in the stomach of young mammals, where it is often referred to as rennin. The cheese making industry relied on extracts from calves' stomachs until the advent of gene technology. Now chymosin can be obtained from modified yeast cultures grown in fermenters.

The techniques of Genetic Manipulation

Gene technology relies on the fact that the genetic language is universal; the 'software' (genes) can be used in any living organism. The difficulty lies in the 'cutting and pasting' of genetic information from one organism to another, and you must first become familiar with three enzymes which are crucial to this process.

Reverse transcriptase

Reverse transcriptase is an enzyme which makes a single strand of DNA from an RNA template. From this strand of DNA, a complementary strand can be assembled with the aid of DNA polymerase. Reverse transcriptase enzyme was identified and isolated from **retroviruses**, a group of viruses which includes the HIV virus. The genetic material of retroviruses is RNA (not DNA), and when retroviruses invade cells their RNA uses reverse transcriptase to make a piece of DNA. This becomes incorporated into the host cell's own DNA and directs the production of new viruses (an example of natural genetic modification of the host cell).

Reverse transcriptase is used in gene technology to make DNA from mRNA. Remember that mRNA is a copy of a single gene and that mRNA of a working gene may be found in the cytoplasm of a cell. It is possible to isolate a gene in the form of mRNA and then to multiply it using a combination of reverse transcriptase and DNA polymerase.

Restriction endonuclease enzymes

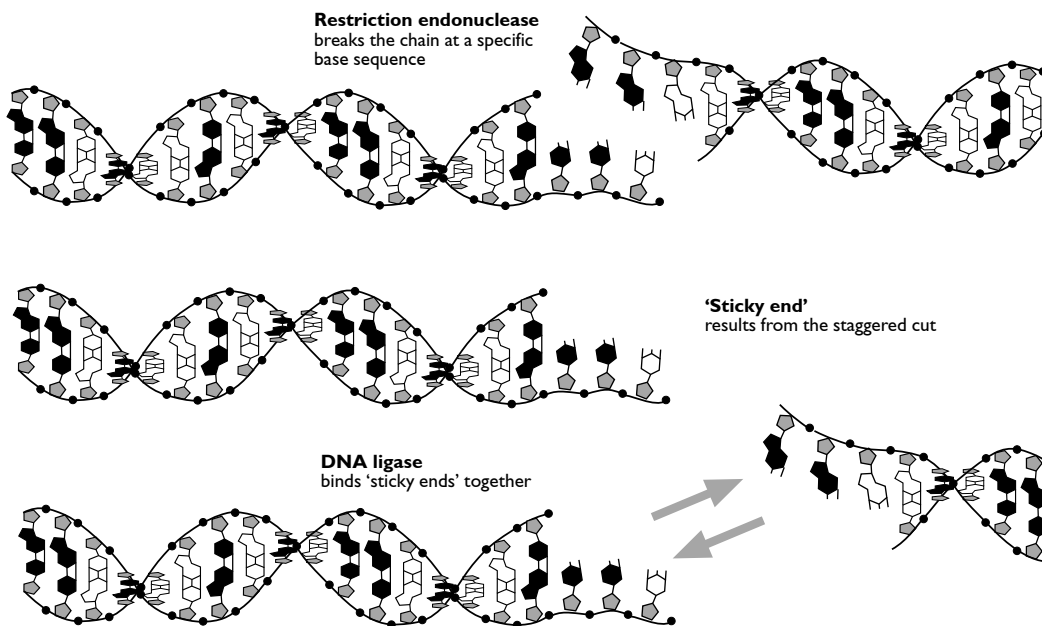
Another vital tool for gene technologists are enzymes called restriction endonucleases (restriction enzymes) which act like scissors to chop up DNA at specific base code sites into smaller polynucleotide fragments. Restriction enzymes are extracted from bacteria. In bacteria they serve to protect the organism from invading viral DNA. In gene technology they are used to cut DNA into fragments which can be separated and isolated. Often the cut leaves '**sticky ends**' which are exploited in another stage of the process.

DNA ligase

To 'ligate' is to join. DNA ligase is a joining enzyme. It is used to stick the sticky ends of DNA fragments together creating new combinations (**recombinant DNA**).

The techniques for transferring genes from one organism to another can be considered in five main stages.

Action of restriction and ligase enzymes



Isolation of the required gene

It is surprisingly easy to extract DNA from living cells as you may already have discovered in the laboratory. Using restriction enzymes and gel electrophoresis it is possible to separate and isolate different DNA fragments, even pure genes. Alternatively, genes can be extracted from cells in the form of mRNA as described above and converted into DNA using reverse transcriptase.

The use of a vector

A vector is a carrier. The most common vectors in gene technology are bacterial plasmids and

bacteriophage viruses, both of which can carry the required gene to the host cell.

Plasmids are rings of DNA found in bacterial cells which are not part of the main bacterial chromosome. They are easily transferred from one bacterial cell to another. Plasmids can be cut at specific sites using restriction enzymes to leave sticky ends. If the same enzyme is used on the isolated gene then the sticky ends will match and the two can be joined using DNA ligase, so that the plasmid contains the recombinant DNA.

Bacteriophages are viruses which infect bacteria. They have a simple ring of DNA which they inject into the bacterial cell. This ring of DNA resembles a plasmid and can be modified in the same way to contain the required gene.

Transformation of the host cells

The vector is now brought into contact with the host cells, in the hope that some of the recombinant DNA might be taken up by the host cells. In order to discover whether or not the host cells have been transformed, **marker genes** (selection genes) are used. These are genes which are engineered into the vector alongside the required gene. They do not harm the host cell in any way but code for products which show up in an easily identifiable form in the host cell, confirming that the process of transformation is complete. One of the more common marker genes codes for antibiotic resistance. The transformed host cells can be isolated by introducing an antibiotic to the culture. All non-transformed cells are killed by the antibiotic, but the transformed cells survive as a result of the antibiotic resistant marker gene.

There has been some concern expressed about the use of antibiotic resistant genes. Opponents of this technique point to the risk of such genes escaping and entering disease organisms. Imagine the potential of a disease-causing bacterium, genetically modified to resist antibiotic treatment.

Multiplication of transformed cells

Once a cell or group of cells has taken up recombinant DNA, the next step is to culture the cells in a suitable growth medium, allowing them to grow and multiply. In the case of transformed bacterial and yeast cells, this can be done on a large scale in industrial fermenters, allowing the product(s) of the new gene to be harvested and purified, as in the case of insulin, cofactor VIII and chymosin.

Questions raised by Gene technology

Gene technology raises many hopes for the future. It should be possible for example to expand the tolerance range and extend the habitat of many crop plants by engineering resistance to salinity, heat, cold, and wind. New varieties will compete better with weeds and be more tolerant of attack from insects and fungal disease. Nitrogen fixation will be enhanced, and renewable energy crops will begin to make a realistic difference to the greenhouse gas levels. Malnutrition may be overcome by staple food crops with a complete complement of essential amino acids, and new drugs will be developed from plant substances.

It also raises a number of questions about the way we want to live. Genetic modification has brought in the age of the designer plant, potatoes exclusively designed for oven chips, square tomatoes, straight bananas, salad vegetables, and fruits of every colour and texture. If there is a market for it, then the plant scientists will be able to engineer it. However, the accidental transfer of new genes into wild organisms could lead to herbicide resistant weeds and antibiotic resistant bacteria. Transgenic plants can also lead to transgenic pests and microbes.

The world population, currently about 5.5 billion, is expected to double to 11 billion by the year 2050. Given that 1 billion are already classified by international agencies as 'poor' and 500 million live in 'absolute poverty', it is clear that food production must be more than doubled in the next 50 years, and this must be achieved on a reducing area of fertile land.

Plant biotechnology, already moving at a rapid pace, offers some hope for the future. Plant biotechnology however, is largely the property of 'First World' independent companies, and 97% of the world's population increase will occur in the developing nations. Furthermore, environmental safety procedures, which evolve with the industry, exist in, and apply only to the countries which have the technology, yet transgenic products now have world wide distribution. A great deal of international cooperation, not to mention finance, will be needed to overcome the problem of safely transferring the products of the new technology to the countries which need them most. The human genome project which is analysing the sequence of bases in human DNA, also raises many problems to be dealt with. Certainly some inherited conditions and tendencies will be better understood, and various treatments developed. On the other hand the potential for abuse is enormous, including refusal of insurance cover to certain groups, employment vetting, immigration control and many others.

◆ CHECKPOINT SUMMARY

- ◆ Isolation of the gene to be manipulated is the first step
- ◆ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself
- ◆ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA
- ◆ Plasmids (circular DNA) are isolated from bacteria
- ◆ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes
- ◆ The genetically modified plasmids are then reintroduced into the bacteria
- ◆ The bacteria multiply and produce the protein coded for by the inserted DNA
- ◆ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene
- ◆ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive
- ◆ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.

AS Module 1

Molecules, Cells & Systems

10.4 Large molecules in the structure and functioning of cells

Biological molecules

Topic Guide

Session:

KEY SKILLS IT3

Check List

- ▼ Most of the important biological molecules are 'organic', which means that they are made up from carbon compounds
- ▼ Not all compounds containing carbon are organic. Exceptions include carbon dioxide and carbonates).
- ▼ Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids,
- ▼ Individual molecules (monomers) can join to form dimers of just two monomers, or longer chains (polymers) of repeating monomers.
- ▼ Polymers are very large molecules, built up by reactions called condensation reactions in which a molecule of water is lost as each sub-unit joins the next.
- ▼ Bonds formed by condensation reactions may be broken down by the reverse process, hydrolysis, which involves the addition of a molecule of water for each bond broken.
- ▼ Condensation and hydrolysis reactions are catalysed by enzymes in the living cell.
- ▼ Under laboratory conditions, in the absence of a catalyst, hydrolysis requires extreme conditions of temperature and pH .

Student Activity

Materials

- Molecular model kit
- Practical Work Sheet:

Tests for substances of biological importance

□ OHT Polymers

Procedure

Carry out the biochemical tests for the major groups of substances of biological importance.

Set work

Tabulate the results from the biochemical tests.

Notes:

AS Module 1

Molecules, Cells & Systems

10.4 Large molecules in the structure and functioning of cells

Carbohydrates

Topic Guide

Session: _____

KEY SKILLS C3.1b: IT3.3

Check List

- ▼ Simple formula for glucose - $C_6H_{12}O_6$
- ▼ Forms straight chain in dry powder form.
- ▼ Forms ring structure in solution.
- ▼ alpha and beta glucose are isomers differing in the 3D arrangement of their atoms.
- ▼ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ▼ Glycosidic bonds are broken by the addition of the elements of water across the bond - a hydrolysis reaction.
- ▼ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ▼ Both being long chains (polymers) of alpha glucose molecules.
- ▼ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix.
- ▼ Starch molecules are relatively insoluble in water.
- ▼ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix.
- ▼ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons which form cellulose fibres.

Student Activity

Materials

- Molecular model of alpha and beta glucose.
- Gravy powder, bunsen, gauze, and beaker with water
- Small tasting samples of glucose, fructose, sucrose in powdered form on sterile filter papers
- OHT Monosaccharides and disaccharides
- OHT Polysaccharides

Procedure

- OHT Monosaccharides and disaccharides recaps structure of alpha and beta glucose

Observe molecular models of glucose.

- OHT Polysaccharides

To illustrate the principle of starch hydration, dissolve the gravy powder in a small volume of water, then heat gently in the beaker (safety glasses etc). Describe how and when it thickens, then discuss why this happens.

'Blind' tasting of fructose, glucose and sucrose.

Put the substances in order of sweetness. Discuss how properties can vary with small molecular differences. (Caution - do not use galactose. Some people are intolerant)

Set work

Make molecular diagrams to show the structure of alpha and beta glucose, maltose, starch and cellulose.

Notes:

AS Module 1

Molecules, Cells & Systems

10.4 Large molecules in the structure and functioning of cells

Proteins

Topic Guide

Session: _____

KEY SKILLS C3.1b

Check List

- ▼ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH_2), a carboxyl acid group (COOH), and a 'R' group, all attached to a carbon atom.
- ▼ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of $\text{CH}_2\text{NH}_2\text{COOH}$.
- ▼ Amino acids combine in a condensation reactions which form a peptide bond.
- ▼ There are about 20 different types of amino acids found in proteins of living organisms.
- ▼ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ▼ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.
- ▼ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins.
- ▼ Hydrogen bonds between adjacent NH_2 and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein.
- ▼ Bonds that form between the 'R' groups determine the tertiary structure.
- ▼ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein.
- ▼ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

Student Activity

Materials

- Molecular models of two amino acids, one with an 'R' group of just one hydrogen atom, and one with a long chain.
- Popper beads.

- ☐ OHT Amino acid structure and peptide bond
- ☐ OHT Secondary and tertiary structures of proteins
- ☐ OHT Structure of haemoglobin

Procedure

Observe 3D models of amino acids.

Combine two to form a peptide bond.

Explain the different R group bonds: hydrogen, ionic, disulphide and hydrophobic (see text)

- ☐ OHTs revise structures.

Set work

In what ways are the structure of Aspartame, insulin, collagen and haemoglobin similar.

In what ways are they different.

Notes:

AS Module 1

Molecules, Cells & Systems

10.4 Large molecules in the structure and functioning of cells

Lipids

Topic Guide

Session:

KEY SKILLS

Check List

- ▼ Triglycerides are composed of three (tri) fatty acids combined by condensation reactions to form ester bonds with glycerol.
- ▼ Fatty acids have long hydrocarbon chains and a carboxyl group (COOH).
- ▼ Saturated fatty acids (and hence saturated fats) have no double bonds in the hydrocarbon chain which is 'saturated' with hydrogen.
- ▼ Unsaturated fatty acids have one double bond.
- ▼ Polyunsaturated fatty acids have two or more double bonds.
- ▼ Lipids include fats, oils, waxes, phospholipids and steroids.
- ▼ Fats (triglycerides) are energy rich storage products in plants and animals.
- ▼ Phospholipids are formed from triglycerides by the replacement of one fatty acid by a phosphate group.
- ▼ Phospholipids form the structural basis of biological membranes by forming a bilayer.
- ▼ The bilayer is formed with their hydrophobic poles adjacent and their hydrophilic poles in contact with the aqueous phase.

Student Activity

Materials

- Range of fats, oils and waxes
- OHT Structure of triglyceride
- OHT Phospholipids and the bilayer

Procedure

Observe the range of fats, oils and waxes.

Tabulate the location and functions of fats, oils, waxes, phospholipids, cholesterol and steroids in living organisms.

Set work

Make notes to explain the structural differences between saturated and non-saturated fats

Notes:

AS Module 1

Molecules, Cells & Systems

10.4 Large molecules in the structure and functioning of cells

Chromatography

Topic Guide

Session:

KEY SKILLS N3.1

Check List

- ▼ Paper Chromatography is used to separate small traces of materials from a mixture.
- ▼ A concentrated spot of the mixture is placed near one end of a piece of chromatography (or filter) paper. The end of the paper is then dipped in a solvent solution such as ethanol and water.
- ▼ As the solvent front moves up the paper, so substances dissolved in it are carried along.
- ▼ The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper.
- ▼ The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a chromatogram, is allowed to dry.
- ▼ By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_F (relative front) value can be obtained.
- ▼ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram.
- ▼ A slightly more sophisticated and faster version of chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above.
- ▼ Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts

Student Activity

Materials

- Filter paper
 - Chloroplast 'pellet' from centrifugation of spinach leaves
 - Solvent mixture of acetone and petroleum ether
 - Tubes
 - Practical Work Sheet:
- Chromatography**

Procedure

Carry out the simple separation of chlorophyll pigments using a safe and supervised procedure.

Practical Work Sheet 4: Chromatography contains more complete instructions.

Set work

Mount your simple chromatography paper strip and label the different pigments identified.

Notes:

Find out the name of an inorganic catalyst, the reaction that it catalyses, and the conditions necessary for the reaction to proceed.

AS Module 1

Molecules, Cells & Systems

10.5 Enzymes as proteins which control biochemical reactions in cells

Factors which affect enzyme activity

Topic Guide

Session: _____

KEY SKILLS C3.2: N3 all IT3.2

Check List

- ▼ pH has an effect on the 3D shape of enzymes and particularly the active site which has an electric charge.
- ▼ Any effect on the active site alters the reactivity of the enzyme. There is an optimum pH the either side of which reactivity drops.
- ▼ Extremes of pH permanently disrupt the active site and thus denature the enzyme.
- ▼ Similar effects are seen with temperature, except low temperatures do not denature enzymes. The rate of enzyme catalysed reactions, like all chemical reactions, are reduced with low temperatures.
- ▼ Optimum conditions of pH and temperature vary with the particular enzyme, which reflects the conditions in which the cell exists.
- ▼ The relative amounts of enzyme and substrate affects the rate of reaction as it affects the formation of the enzyme substrate complex. Only one can be rate regulating or limiting at any one time.
- ▼ Rates of disappearance of substrate are proportional to the rate of reaction.
- ▼ Rates of formation of end-product(s) are proportional to the rate of reaction.

Student Activity

Materials

- Practical Work Sheet:
The effect of temperature on the activity of catalase
- OHT Temperature, pH, enzyme and substrate concentration

Procedure

Practical Work Sheet: The effect of temperature on the activity of catalase provides full practical details.

Set work

Plot the curve for the rate of a typical chemical reaction against temperature from 0 - 100°C

On the same axes plot the rate of an enzyme catalysed reaction with an optimum temperature of 40°C.

Explain the relationship shown by the two curves.

Notes:

AS Module 1

(Biology and Human Biology)

Molecules, Cells and Systems

10.4 Large molecules in the structure and functioning of cells

Biological molecules

Carbohydrates

Proteins

Lipids

Biochemical tests using Benedict's reagent for reducing sugars and for non-reducing sugars after acid hydrolysis. Iodine/potassium iodide solution for starch. The biuret test for proteins. The emulsion test for lipids.

Biochemical tests for substances of biological importance

Introduction

A standard series of procedures are used to test for a variety of biological molecules. These tests are traditionally known as 'food tests', but here the basic test methods are performed on a standard solution of each type of substance.

Method - Benedict's test for reducing sugars

- ▼ Put on a pair of safety glasses. Collect a 500cm³ beaker and fill it about half full with tap water. Place it on a tripod and gauze over a Bunsen burner and bring the water to the boil.
- ▼ Place a small quantity of reducing sugar solution (about 1-2 cm³) in a boiling tube. Add 2 cm³ of Benedict's reagent.
- ▼ Place the boiling tube in the waterbath and boil the tube and its contents for 3-4 minutes. Remove the tube from the waterbath using test-tube tongs and place it in a test-tube rack.

If the sample contains reducing sugars, a precipitate will be produced, and the colour will change from blue through green and yellow to brick-red, depending on the amount of sugar present. This test can thus be used to give an estimation of the amount of sugar present, provided the quantities of sugar solution and Benedict's reagent used are the same each time. A small amount of sugar will turn the solution green, larger amounts yellow and considerable quantities will turn it brick-red.

Method - Benedict's test for non-reducing sugars

This test should be done if the previous test for reducing sugars gives a negative result.

- ▼ Place a fresh sample of sugar solution (1-2cm³) in a boiling tube and add 1cm³ dilute hydrochloric acid. Place the boiling tube in the waterbath and boil it for 1-2 minutes.
- ▼ Remove the test-tube from the waterbath using tongs and place it in a test-tube rack. Allow to cool for a couple of minutes. Using a spatula, add small quantities of sodium hydrogen carbonate powder, until the mixture stops fizzing. (This will probably take about 3 small spatulas full: the sodium hydrogen carbonate neutralises the acid)
- ▼ Add 2cm³ Benedict's reagent to the boiling tube and put it back in the waterbath, using the tongs. Boil it again for 3-4 minutes.

If non-reducing sugars (e.g. sucrose) were present in the original sample, the acid will hydrolyse them to monosaccharides, which are all reducing sugars: they will then give a green/yellow/brick-red precipitate with Benedict's reagent.

Method - Samples containing both reducing and non-reducing sugars

If you suspect that a sample contains both reducing and non-reducing sugars:

- ▼ Test with Benedict's reagent and obtain the precipitate as described above (using double quantities of sample and reagent)
- ▼ Filter the tested sample through filter paper, and collect the filtrate.
- ▼ Test the filtrate with Benedict's reagent.
- ▼ Continue this process, using the filtrate each time, until you no longer get a positive result with the Benedict's test.
- ▼ Add 1cm³ dilute hydrochloric acid to the remaining filtrate and boil it to hydrolyse any non-reducing sugars it contains. Neutralise the mixture and re-test with Benedict's reagent.
- ▼ If a non-reducing sugar was present in the original sample there will be a positive result once again.

Method - Iodine in potassium iodide solution test for starch

- ▼ Place a small sample of starch solution on a spotting tile or in a test-tube.
- ▼ Add a few drops of iodine in potassium iodide solution.

The starch will combine with the iodine to give a deep blue-black colour. This test is very sensitive, so only tiny quantities are needed. The colour will fade after a while.

Method - Emulsion test for lipids

This test uses the fact that alcohols will dissolve lipids, but water will not.

- ▼ Place a small sample of lipid in a test-tube. Add $1\text{--}2\text{cm}^3$ of alcohol and shake the test-tube thoroughly for a few minutes. Place it in a test-tube rack and allow it to settle.
- ▼ Collect a clean test-tube and half fill it with tap water. Carefully pour the alcohol layer from the sample into the water.

The lipids will dissolve in the alcohol as the sample is shaken. When the solution is poured into the water, the alcohol will dissolve in the water, but the lipids will come out of solution and form fine droplets. Thus if there are lipids in the sample, they will form a cloudy white emulsion in the water.

Method - Biuret test for proteins

- ▼ Place 2cm^3 of protein solution in a test-tube. Add 2 cm^3 of dilute potassium hydroxide solution, then add 0.5cm^3 of dilute copper sulphate solution, a drop at a time. Shake the test-tube gently, then leave it in the test-tube rack for a few minutes.

If protein is present, a purple colour will develop after a few seconds (s). If there is no protein, the solution will remain very pale blue from the copper sulphate solution.

These methods may be used for testing materials in a laboratory, or for testing foodstuffs to analyse their contents.

Teaching Notes

For the emulsion test, either ethanol or iso-propanol will work, provided it is a strong solution (at least 70%).

The only problem with the standard tests is doing the emulsion test on milk, in which the emulsion effect is masked. The Sudan III test for lipids may be more appropriate for this situation, as it gives a clearer result. If milk is shaken up with Sudan III and methylene blue and allowed to settle, the red Sudan III will separate out in the fatty layer at the top, and the methylene blue in the aqueous layer at the bottom.

The test for reducing sugars can be made quantitative by carrying out the Benedict's test on a series of known concentrations of glucose solution, to produce a colour series, then testing some unknowns to identify where they fit in the series. This could be introduced as a problem for the students, to provide experience in planning experiments.

Clinistix or equivalent can be used to test for different glucose concentrations.

Once the tests are understood, the techniques could be used to analyse mixtures. This could be made more interesting by suggesting that they are testing foodstuffs for a supermarket firm who have been told that some of their foods have been spiked, e.g. try adding starch to the milk, or injecting some oil into oranges, for example.

Alternatively, there is a good variation published in *Journal of Biological Education*, (Roger Law, 1995, "An A-level food test investigation", *JBE*, Volume 29, p251-252), which uses other tests as well, to analyse mock "stomach contents" from two animals. The idea is to decide whether they are carnivorous or herbivorous. This test uses some solutions which are a bit more risky than the usual ones (Schultz' reagent for cellulose, for example.) However, it can make an enjoyable change.

Requirements per student

Boiling tubes
Test tubes
500cm³ beaker
Bunsen burner, tripod and gauze
Test-tube rack
Test-tube tongs
Spotting tile
Small spatula
Filter funnel and filter paper
Safety glasses
Small measuring cylinders or dropping pipettes

Benedict's reagent
Dilute hydrochloric acid
Sodium hydrogen carbonate powder
Iodine in potassium iodide solution
70-80% alcohol solution
Biuret 1% copper sulphate solution
Biuret 15% potassium hydroxide solution

Standard solutions of sucrose (5%), glucose (5%), Starch (1%), albumin (5%) and a small sample of cooking oil.

continued . . .

Safety Considerations

The following chemicals need to be handled carefully:

Dilute hydrochloric acid – corrosive

Iodine in potassium iodide solution – irritant

80% alcohol – highly flammable

Biuret potassium hydroxide – corrosive

Safety glasses should be worn for the Benedict's test at least: you may prefer students to wear them all the time.

The Benedict's reaction should be boiled in a waterbath – never allow students to heat the tube directly in the Bunsen flame.

Normal precautions for handling hot apparatus should be observed.

AS Module 1

(Biology and Human Biology)

Molecules, Cells and Systems

Large molecules in the structure and functioning of cells

Chromatography

The technique of chromatography to illustrate how molecules may be separated and identified. The calculation and use of R_f value.

Two way chromatography.

The technique of chromatography

Introduction

Chromatography is a technique used to separate and analyse which molecules are present in a mixed extract from living material. It depends on the use of organic solvents, which dissolve the molecules concerned. These solutions are allowed to move up a thin layer of inert material, such as paper or a thin layer of silica gel, carrying the molecules with them. The distance the molecules move is related to the size and shape of the molecule, as well as their relative solubility in the solvent and the water associated with the layer of inert material.

For each combination of molecule and solvent, the distance moved relative to the solvent alone is characteristic, and can be used to identify the molecule. The value is known as R_f (Relative Front) and is calculated thus:

$$R_f = \frac{\text{Distance moved by molecule}}{\text{Distance moved by solvent}}$$

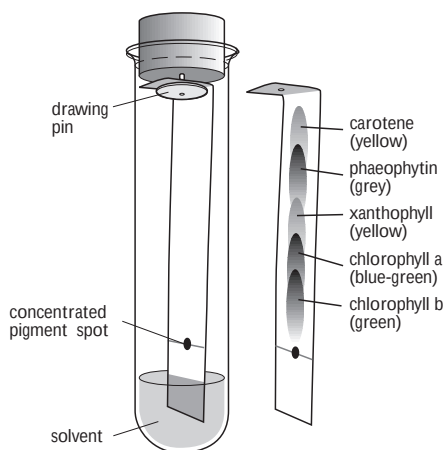
Method

- ▼ Cut a piece of chromatography paper (or filter paper) about 1.5cm wide, and long enough to reach the bottom of the boiling tube. Fold the top 1cm over, and make sure the paper will fit into the boiling tube, with the folded edge at the top, the end nearly reaching the bottom, and the sides NOT touching the sides of the tube. If necessary, trim it to fit.
- ▼ Rule a pencil line (not pen!) across the paper about 2.5 cm from the bottom. Mark the centre of the line with a cross. Write your name at the top of the strip in pencil.
- ▼ Collect a handful of plant material, and cut it up quite finely with a sharp knife. Place 5 cm³ of propanone in the mortar, and grind up the plant material in the mortar, using the pestle and a small amount of sand, (or use a liquidiser). The green pigments should dissolve into the propanone. As you grind up the plant material, remove any tissue that is well ground, and add more, but do not add any more propanone: you need to get as concentrated a solution as possible.
- ▼ Once you have a concentrated solution, filter the contents of the mortar through a piece of gauze in a filter funnel. Discard the solid materials.
- ▼ Put a small amount of solvent (to a depth of not more than 1.5 cm) in the bottom of the boiling tube. Put the bung in the tube and leave it on one side so that the air in the tube can saturate with the solvent vapour.

continued

- ▼ Using a piece of very fine capillary tubing or the head end of a pin, place a drop of the extract on the cross in the middle of the pencil line of the paper strip. Use a hair dryer to dry the drop, then put another drop in the same place. Repeat this process until you have a small area of concentrated pigment. Be patient with this: you will get a much better result if you let each drop dry properly before you add the next. You should add at least 5-6 drops.
- ▼ Use a drawing pin stuck into the base of the bung, or a split bung, to suspend the paper in the boiling tube, so that the end of the paper reaches into the solvent, but the spot of pigments is not in the solvent. Adjust the amount of solvent if necessary.
- ▼ Leave the boiling tube in a rack to allow the solvent to move up the filter paper. When the solvent has nearly reached the top of the strip of paper, remove the bung and detach the paper. Draw a pencil line on the paper to mark the level reached by the solvent, then leave it on the bench (or in a fume cupboard) to dry.
- ▼ Measure the solvent distance, and the distances moved by the spots of pigment. Identify the pigments by their colour and R_f values, using the information given in the table below. If you have produced a good concentration of pigments in the extract and spot, you should be able to identify all five pigments.

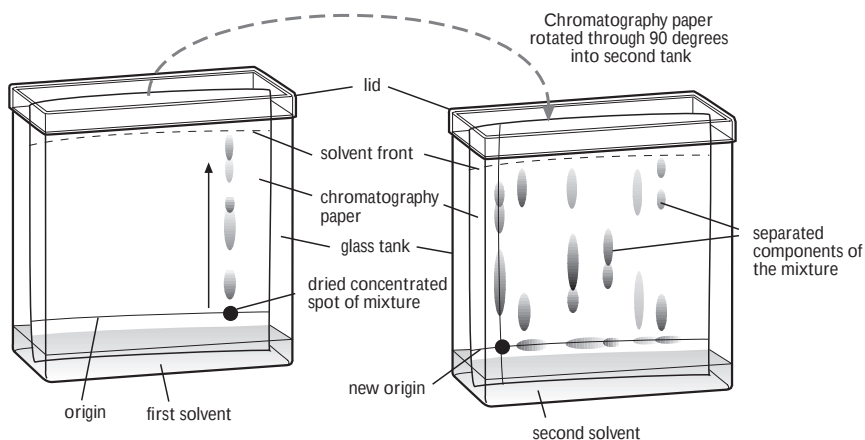
Pigment Name	R_f value	Colour
Carotene	0.95	Yellow
Phaeophytin	0.83	Yellow-Grey
Xanthophyll	0.71	Yellow-Brown
Chlorophyll a	0.65	Blue-Green
Chlorophyll b	0.45	Green



Method - Two way chromatography

Two way chromatography is used to make further separations of substances that may not have been separated sufficiently on the first run of a chromatography investigation.

The chromatography paper from the first run is turned by 90° and re-run as shown in the diagram. Details are provided with Chromatography kits, and are not repeated here as no new principles are involved.



Teaching Notes

The solvent is made from 1 part 90% Propanone (Acetone) to 9 parts Petroleum ether (alternatively 12 parts: 100 parts)

The plant material needs to be as green as possible! Nettle leaves work well, and can be handled easily with gloves. Alternatively, spinach leaves also give a good result. As a possible extension, or for a student project, it might be interesting to try a different coloured leaf, such as red cabbage, although the R_f values for any extra pigments may be difficult to find.

One important factor in getting the practical to work well is to get the chlorophyll extract as concentrated as possible: the students need to spend time over this, and grind up quite a lot of plant material in as little propanone as they can. The other stage at which patience is needed is the preparation of the pigment spot. They must let each drop dry thoroughly, before adding the next, and they need to add at least 5-6 drops. Using a hair dryer speeds up the process. To keep the evidence of the experiment; after allowing the filter paper to dry, it should be sealed under Sellotape so the pigments do not fade.

If you have access to thin layer plates, they tend to work better than paper sheets, but are more complex to set up. If you do use thin layer plates, the R_f values will be different from those given here.

Requirements per student

Filter or chromatography paper
Scissors
Boiling tube with a rubber bung
Drawing pin
Ordinary pin or piece of fine capillary tubing (especially fine chromatography droppers are available)
Pestle and mortar or liquidiser
Sand for grinding
Pencil and ruler
Sharp knife
Cutting tile
Filter funnel
Piece of gauze, about 12 cm square
50cm³ beaker
Test-tube rack
5cm³ propanone
10cm³ solvent
Plant material, e.g. nettles, spinach etc.

Safety Considerations

Both the propanone and the solvent are highly flammable and need to be handled with care. The lesson should be run in a well-ventilated room, and all naked flames should be extinguished. Ideally the strips should be dried in a fume cupboard.

If you use nettle leaves, they should be handled wearing disposable rubber gloves of the surgical type or ordinary rubber gloves, these will be better than the very thin film gloves. Once crushed or cut up, the nettles can be handled with bare hands.

AS Module 1

(Biology and Human Biology)

Molecules, Cells and Systems

Enzymes are proteins which control biochemical reactions in cells

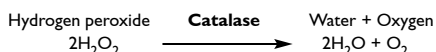
Factors which affect enzyme activity

Description and explanation of the effects of temperature, pH, substrate and enzyme concentration on enzyme activity.

An experiment to investigate the effect of temperature on the activity of catalase

Introduction

Catalase catalyses the breakdown of hydrogen peroxide to oxygen and water.



The rate of production of oxygen can be measured, and gives a measure of the rate of the reaction.

Catalase is produced in cells to prevent the accumulation of hydrogen peroxide, which is toxic.

In this experiment potato tissue is used as a source of catalase.

The effect of temperature on the rate of this reaction will also be investigated.

TAKE CARE with the HYDROGEN PEROXIDE - wear safety glasses and try not to get any on the skin, wash it off immediately if you do.

Read the method sheet through carefully before you start.

Method

▼ There are various pieces of apparatus that you can use to collect and then measure the volume of oxygen gas that will be evolved when the catalase in the potato tissue reacts with the hydrogen peroxide.

a The oxygen gas can be collected over water in an inverted measuring cylinder.

b The oxygen can be collected over water in the inverted barrel of a 20 cm³ syringe.

c The oxygen can be collected and measured in a manometer tube filled with coloured fluid, linked to the reaction vessel. You will be advised which method(s) to use.

The details below relate mainly to the 'inverted measuring cylinder' method.

▼ Using a cork borer a little smaller in diameter than the boiling tubes, cut some long potato cylinders. Then using a knife (scalpel) carefully cut the potato cylinder into discs about 1mm thick.

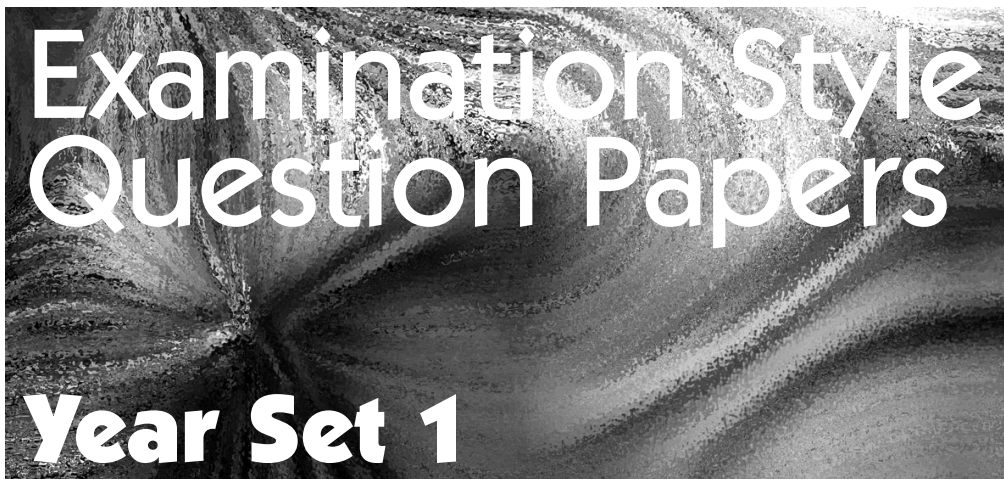
▼ Place the discs into a few cm³ of pH7 buffer in a watch glass to keep them moist. You will need 50-60 discs.

▼ Using a boiling tube as the reaction vessel, place about 10 potato discs into the tube and just cover them with a measured volume of pH7 buffer.

continued

For **AQA** Specifications '**A**'
Advanced Subsidiary

Biology or Biology (Human)



Examination Style
Question Papers

Year Set 1

AS Unit 1 - Molecules, Cells & Systems

AS Unit 2 - Making Use of Biology

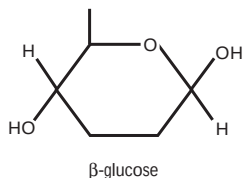
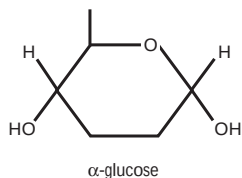
AS Unit 3 - Pathogens & Disease

Complete with Assessment Grids & Mark Schemes

FELTHAM PRESS

Question 3

- a) The structure of alpha glucose and beta glucose is shown below:



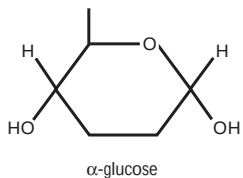
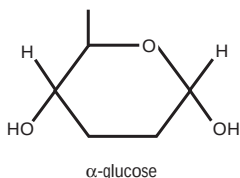
- i) The short-hand convention used in drawing organic molecules in this way omits the carbon atoms. Indicate on one of the diagrams above where the 6 carbon atoms of glucose are to be found

(1)

- ii) Describe how the two molecules shown differ in structure.

(1)

- b) Two alpha glucose molecules are shown below:



- i) Indicate on the diagram how the disaccharide maltose would be formed.

(1)

- ii) What type of reaction is this known as?

(1)

- iii) Name the chemical bond formed in this way.

(1)

- iv) Name and describe the type of reaction that would result in the two alpha glucose molecules being regained from the maltose molecule.

(2)

Total 7

Question 4

- a)** Give a definition of the term catalyst.

(1)

- b)** Name an enzyme and the reaction that it catalyses.

(1)

- c** What is meant by the term 'enzyme specificity'

(1)

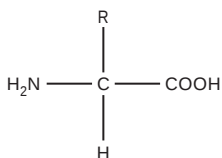
- d)** Enzymes are denatured by extremes of pH. Explain what is meant by the term 'denatured'

(2)

Total 5

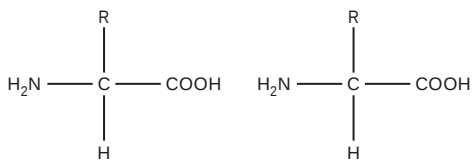
Question 1

The structural formula of an amino acid is shown below.



- a) There are about 20 different types of amino acid to be found in proteins of living organisms, explain how the structural formula shown above applies to them all even though they are different.

(2)



- b) On the diagram below show how two amino acids can combine.

(2)

- c) Name the type of reaction that results in the combination shown in section (b).

(1)

- d) Explain how only about 20 different types of amino acids can form the huge variety of proteins found in living organisms.

(3)

Total 8

Question 4

A solution of unknown composition is tested with Benedict's solution and a positive result is obtained.

- a)** Describe a positive result with the Benedict's test.

(2)

- b)** Explain why it would be wrong to deduce that the positive Benedict's test indicates that glucose is present.

(3)

- c)** Explain why a solution of sucrose must undergo acid hydrolysis before a positive result for the Benedict's test can be obtained from it.

(3)

Total 8

Question 5

- a) Give the names of two proteins found in the body.

(1)

- b) Describe the Biuret test for proteins, and the appearance of a positive result.

(2)

- c) Explain how the shape of the three dimensional tertiary structure of an enzyme is essential for its role as a biological catalyst.

(3)

Total 6